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SYNTHESIS OF TELOMERASE INHIBITORS BASED ON POLYOXAZOLES

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Abstract

This thesis focuses on the synthesis of compounds expected to inhibit telomerase and provide potential for the treatment of cancer. Telomerase is a reverse transcriptase enzyme, which codes for telomeres and define the ends of chromosomes. Abnormal telomerase activity occurs in 85% of cancer cells and consequently has gained considerable interest as a target for cancer therapy. A natural product known as telomestatin can target G-quadruplexes and is shown to be very potent with activity at 5 nM. The aim of this thesis was to synthesise analogues of Telomestatin which contain a polyoxazole macrocycle.

Chapter 1 discusses the enzyme telomerase and describes compounds which are able to inhibit its activity; a literature survey on 2, 4-disubstituted oxazole chemistry is also described.

Chapter 2 describes the attempted synthesis of a dipyridyltrisoxazole and a dipyridyl macrocycle. The synthetic approach involved the Hantzsch method to form oxazoles from amides and bromoketone. The Hantzsch method proved largely ineffective for obtaining the required oxazoles. A second approach involving the Willams-Wipf reaction was carried out to synthesise oxazoles. The procedure involved the cyclisation of L-serine methylester derived compounds with diethylaminosulfur trifluoride, followed by an oxidation reaction with 1,8-diazabicyclo[5.4.0]undec-7-ene and bromotrichloromethane. The Williams-Wipf approach helped to synthesise the half fragment required for the macrocycle.

Chapter 3 involved the synthesis of a tetraoxazolylbipyridyl system. The Williams-Wipf approach was successful in delivering the half fragment of the desired system. However, the palladium cross coupling was ineffective in forming the tetraoxazolylbipyridyl system.

Chapter 4 the aim was to synthesise a symmetrical octaoxazole ring system. The Willams-Wipf approach helped to develop the core structure to telomestatin which consists of five consecutive oxazole rings. A novel hepta oxazolyl ring system was also synthesised; however, owing to insolubility of key intermediates the desired target could not be made.

Acknowledgements

First of all I would like to thank Professor Charles M. Marson for giving me the opportunity to undertake this project which I really enjoyed. I thank him for his advice and enthusiasm throughout the three years. I would also like to thank the Marson group past and present, the technical staff in particular Abil, John, Lisa who were extremely helpful.

Finally, I would like to thank my family, especially my mum and Mehrdad for being there always.

Abbreviations

A	Adenine
Ac_2O	Acetic anhydride
Anal.	Analytical
Bcl-2	B-Cell lymphoma 2
Bn	Benzyl
BnBr	Benzyl bromide
Boc	Butyloxycarbonyl
BOP	Benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate
δ	Chemical shift
C	Cytosine
Cbz	(benzylcarboxy) carbamate
CD	Circular dichroism
CDK	Cyclin-dependant kinases
CSA	Camphorsulfonic acid
$Cu(OTf)_2$	Copper(II) triflate
d	doublet
DAST	Diethylamino sulfurtrifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
Deoxo-Fluor^{TM}	Bis-(2-methoxyethyl)aminosulphur trifluoride
dGTP	2'-deoxygyuanosine 5'-triphosphate
DIEA	Diisopropylethylamine
DMF	Dimethylformide
DNA	Deoxyribonucleic acid
DnaA	DNA replication initiation factor
EDCI	1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide
EI	Electron impact
equvi.	Equivalent
FAB	Fast atom bombardment
G	Guanine
G1	Gap 1
G2	Gap2

h	hours
HATU	2-(1H-7-Azabenzotriazol-1-yl)-1, 1, 3, 3-tetramethyl uranium hexafluorophosphate methanaminium
HDAC	Histone deacetylase
HMQC	Heteronuclear multiple-quantum coherence experiment
HOBT	*N*-Hydroxy benzotriazole
hTERT	Human telomerase catalytic sub-unit gene
Hz	Hertz
IBCF	Isobutyl chloroformate
IC_{50}	Half the maximal inhibitory concentration
J	Spin-spin coupling constant
m	multiplet
Me	Methyl
NBS	*N*-Bromosuccinimide
NIDDM	Non-Insulin Dependant Diabetes Mellitus
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
PCR	Polymerase chain reaction
p-TSA	*para*-Toluenesulfonic acid
q	quartet
RNA	Ribonucleic acid
RPMI 8402	Cell culture made from T-cells
RTER	Telomerase reverse transcriptase
s	singlet
S	Stationary
SAR	Structure activity relationship
T	Thymine
TBDPSCl	Tributyldiphenylsilyl chloride
TBSCl	Tributylsilyl chloride
TEP1	Telomerase associated protein 1
TESCl	Triethylsilyl chloride
TETA	Triethylene tetraamine
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran

TIPSOTf	Triisopropylsilyltriflate
TsCN	Tosyl nitrile
TMANO	Trimethylethylamine *N*-oxide
TMSCl	Trimethylsilyl chloride
TMSOTf	Trimethylsilyl triflate
TRAP	Telomerase repeat amplification
TsCl	Tosyl chloride
TTP	Thymidine triphosphate

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Chapter 1

1.0 Cancer and Inhibitors of Telomerase

1.1 Objectives

This thesis will focus on the synthesis of compounds expected to inhibit telomerase and hence with potential for the treatment of cancer. Abnormal telomerase activity occurs in 85% of cancer cells and consequently has gained considerable interest as a target for cancer therapy. Chapter one will focus on cancer and its treatment, followed by a detailed account of telomerase and a literature survey of oxazole systems found in nature.

1.2 Cancer

Cancer is a disease which is the cause of most deaths in the UK, one third of the population being affected by it at some time in their lives.[1] Cancer involves unregulated cell growth, these cells can act on normal cells to destroy them.

Cancers form as a result of an accumulation of cancer cells in the form of lumps known as tumors. Tumors that arise between epithelial sheets found in inner and outer surfaces in the body are known as carcinomas. Tumors found in connective tissues are termed sarcomas. Leukaemia and lymphomas are a result of circulating tumors found in bone marrow, and lymph nodes. The disease follows a sequence of events which include healthy cells being converted to cancer cells that can proliferate and invade other nearby tissue. Finally, the cells metastasise, which involves liberation of the cells from the tumor mass and migration to other parts of the body. Uncontrollable division of cells will ultimately lead to death, if no treatment is successful.

Chapter 1

Much time and research has been spent trying to understand cancer and what causes it. It is believed that environmental factors such as an over-exposure to ultra-violet light from the sun can be a cause of cancer, as can be radioactive materials and X-rays. Certain compounds or mixtures termed carcinogens,[2] can also lead to cancer; they include tar found in cigarettes, charred food and additives such as phenylalanine found in fizzy drinks. Biological factors including oncogenes from viruses that attack the healthy host can transmit a cancer-causing gene formed as a result of DNA mutations or chromosomal translocation

Those affected by cancer show certain symptoms which often may include the following: lumps around a particular area of the body, bleeding, change in body weight, change in bowel movement, feeling fatigue and nauseous. Blood tests and scans are used to confirm the presence of the disease.

Treatments available for cancer include chemotherapy, radiation therapy and surgery. Chemotherapy involves giving drugs to the patient to kill the cancer cells. Dire side effects can develop from taking the medication such as fatigue, nausea and improvement of the immune system since healthy cells are also targeted by the therapy. The success of the therapy depends in part on the stage of development of the cancer.[2] Hence, higher success rates are normally found in the early stages of cancer.

Many different targets for cancer treatment have been and are being investigated, especially inhibition of enzymes including: histone deacetylase inhibitors (HDAC), inhibitors of Bcl-2 and telomerase inhibitors.

1.3 Structure of DNA

DNA molecules are found inside the nucleus of a cell. A process known as transcription occurs in cells where information is transferred from the nucleus via messenger RNA which is the complimentary base sequence of single-stranded DNA. The translation of the nucleotide sequence contained in mRNA provides a code for protein synthesis. The length of DNA contains the instructions for making a single protein or polypeptide known as the gene that can code for a sequence of amino acids that comprise a protein.

The code in DNA consists of four bases, adenine (A), thymine (T), guanine (G) and cytosine (C), and one of the two strands is named as the reference strand, adenine bonding with thymine, and guanine with cytosine. During transcription, bases are always read in the same direction.

Genetic information contained in the DNA molecule (Fig 1) can be transmitted to the next generation by mitosis during cell cycle.

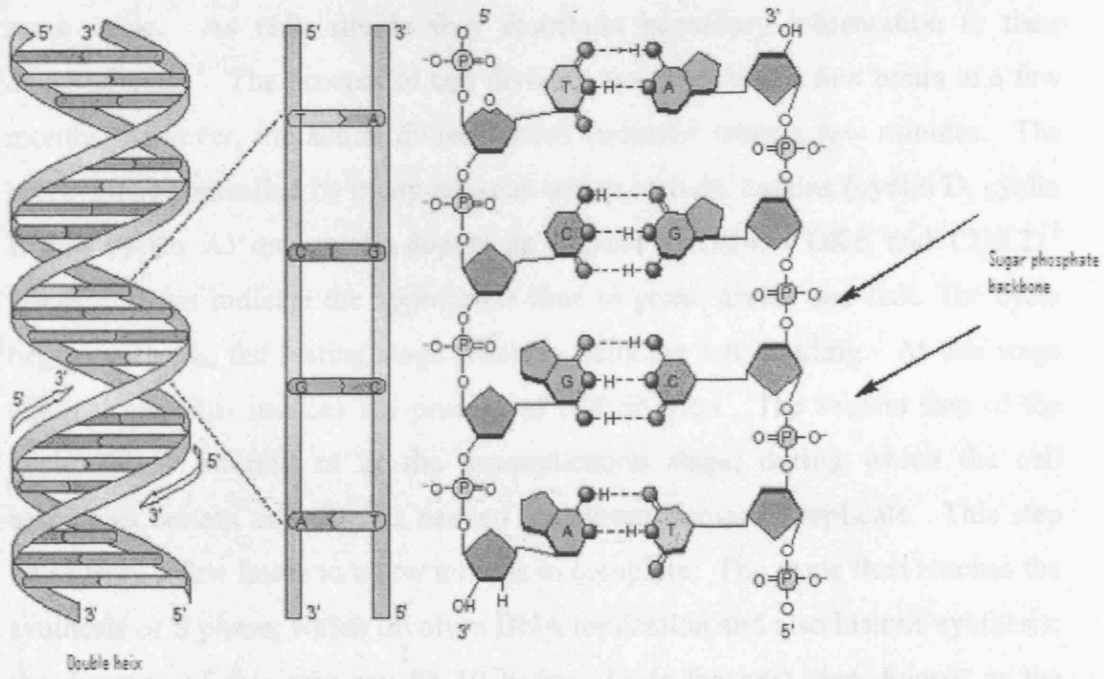


Figure 1. Structure of DNA

1.4 Cell Cycle

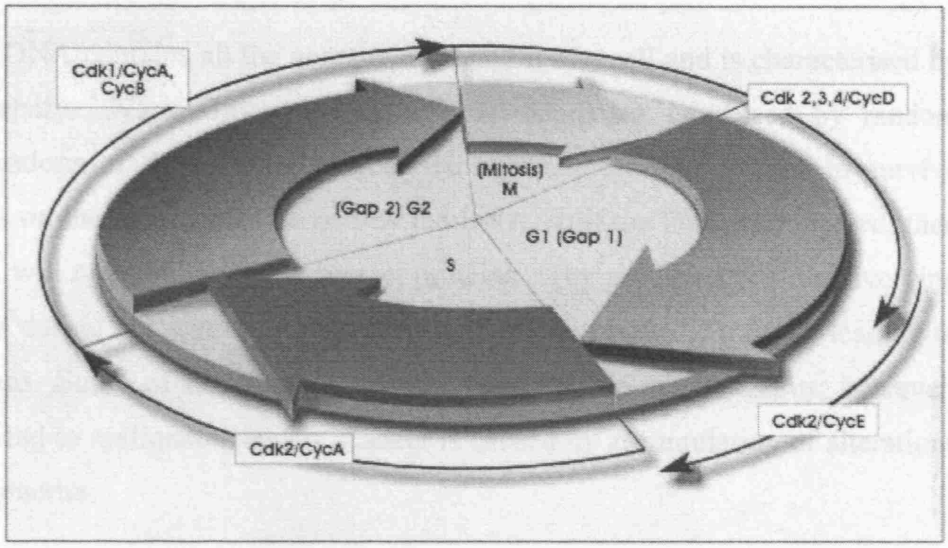


Figure 2. The cell cycle[3]

The cell cycle involves a sequence of events by which a cell grows to develop and divide, forming new daughter cells which then go on to carry out the same cycle. As cells divide they distribute hereditary information to their daughter cells.[4] The process of cell division can take from a few hours to a few months; however, the actual division itself (mitosis) takes a few minutes. The cell cycle is controlled by many proteins which include: cyclins (cyclin D, cyclin E and cyclin A) and cyclin-dependant kinases (CDK4, CDK6 and CDK2).[5] These proteins indicate the appropriate time to grow, divide and halt. The cycle begins with G_0, the resting stage whereby cells are not dividing. At this stage external stimulus induces the process of cell division. The second step of the cycle, G_1 is referred to as the pre-replication stage, during which the cell assembles certain constituents needed for chromosomes to replicate. This step takes from a few hours to a few months to complete. The cycle then reaches the synthesis or S phase, which involves DNA replication and also histone synthesis; the duration of this step can be 10 hours. G_2 is the next step, known as the postmitotic stage and lasts about two hours. Normal metabolism takes place here, allowing the cells to make proteins for cell growth. The cells then go

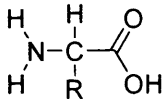
through the same sequence of events to form more cells. Cancer treatment focuses on controlling one or more of those stages.

DNA contains all the genetic information of a cell and is characterised by its diversity. Variability in DNA heterocyclic bases is caused by random modifications of the DNA sequence. However, the ability of cell to survive depends on the instructions carried by the DNA, so if the DNA is damaged, then the cell will not synthesise the correct proteins. The problematic time involving DNA is during replication because errors in reading bases can occur leading to mutations. Some of the random modifications can be advantageous; however, others lead to malignant cancers. Cancer is caused by accumulation of alterations in the genome.

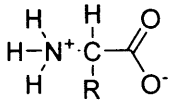
1.5 Amino acids

All proteins are made from polymers of various amino acid subunits to form complex structures. There are twenty naturally occurring amino acids and all occur in the *laevo* configuration except glycine which has no stereocentre.

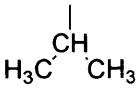
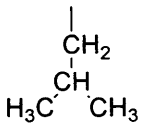
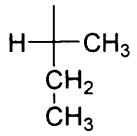
Each amino acid contains an amino group, a carboxyl group and a different R group. When an amino acid is dissolved in water it can dissociate so that the acidic carboxyl group loses a hydrogen atom and the amino group gains a hydrogen atom due to its basicity, making it a Zwitterion. The different R groups present determine the physical properties of the structure. Alanine, valine, leucine, isoleucine, phenylalanine, tryptophan are all hydrophobic, containing hydrocarbon side chains. Aspartic acid, aspargine, glutamine, tyrosine and cysteine are some of many amino acids which are hydrophilic in nature hence, quite water-soluble. Cysteine contains a highly nucleophilic thiol which can react with another cysteine amino acid to form disulfide linkages that permit for complex bridged protein structures (fig 3).



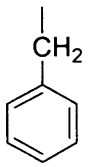
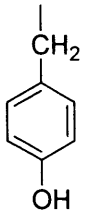
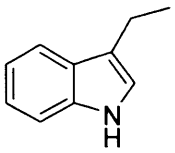
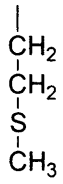
Amino acid



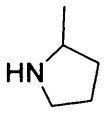
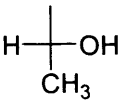
dissociated form

R=     

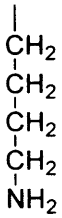
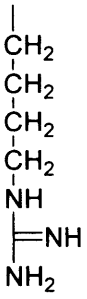
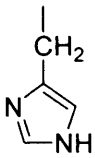
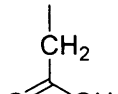
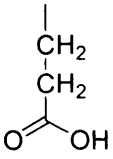
Glycine	Alanine	Valine	Leucine	Isoleucine
(Gly, G)	(Ala, A)	(Val, V)	(Leu, L)	(Ile, I)

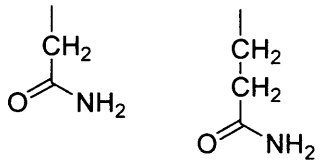
Phenylalanine	Tyrosine	Tryptophan	Methionine
(Phe, F)	(Tyr, Y)	(Trp, W)	(Met, M)

Proline	Serine	Threonine	Cysteine
(Pro, P)	(Ser, S)	(Thr, T)	(Cys, C)

Lysine	Arginine	Histidine	Aspartine	Glutamine
(Lys, K)	(Arg, R)	(His, H)	(Asp, D)	(Glu, E)



Asparagine (Asn, N) Glutamine (Gln, Q)

Figure 3. Amino acids

Proteins are made from amino acids linked by peptide bonds which are strong covalent bonds. The amino group attacks the acid group to form a bond via a condensation reaction with loss of water. Two linked amino acid units are termed dipeptide; a third amino acid sequence is termed tripeptide. Longer amino acid polymer chains are known as polypeptides; the chain contains an amino and carboxyl terminus.

Scheme 1. Peptide formation

Proteins are usually a sequence of 100 to 10,000 amino acids which depending on their inter-and intramolecular bonding, can form different structures. Linear sequences of amino acids form a primary structure of alpha helixes that take the shape of a telephone cord. The structure is held together by hydrogen bonding between N-H and C=O groups. An example of a secondary structure protein is that of keratin which is found in a strand of hair.

Proteins with a longer amino acid chains can form more complex structures. When two or more polypeptide chains lie side by side, hydrogen bonding is crossed linked to form beta-pleated sheet structure.[4] Such protein structures are found in silk. Bonds involved in these protein structures involve electrostatic forces, van der Waals, and hydrophobic interactions.

Proteins can fold together and form a tertiary structure which is highly complex. As well as hydrogen bonding, disulfide linkages can also take place

between cysteine residues to form a three-dimensional globular shape. Ribonuclease is made up of a tertiary structure which helps it to bind to an RNA molecule and splits RNA apart. Two to three polypeptide chains that form tertiary and secondary proteins can go to form quaternary structures. The three dimensional shape is held by weak bonds, an example of which is haemoglobin.

1.6 Enzymes

Studies on enzymes have been carried out for more than a hundred years. Findings have proved that enzymes play a key role in biological reactions in the body; the main reason for this is due to specificity. An enzyme reacts with one type of substrate (the reactant) to form the desired product. An enzyme acts as a biological catalyst owing to its ability to lower the activation energy of a reaction without the enzyme being consumed or altered in any way.

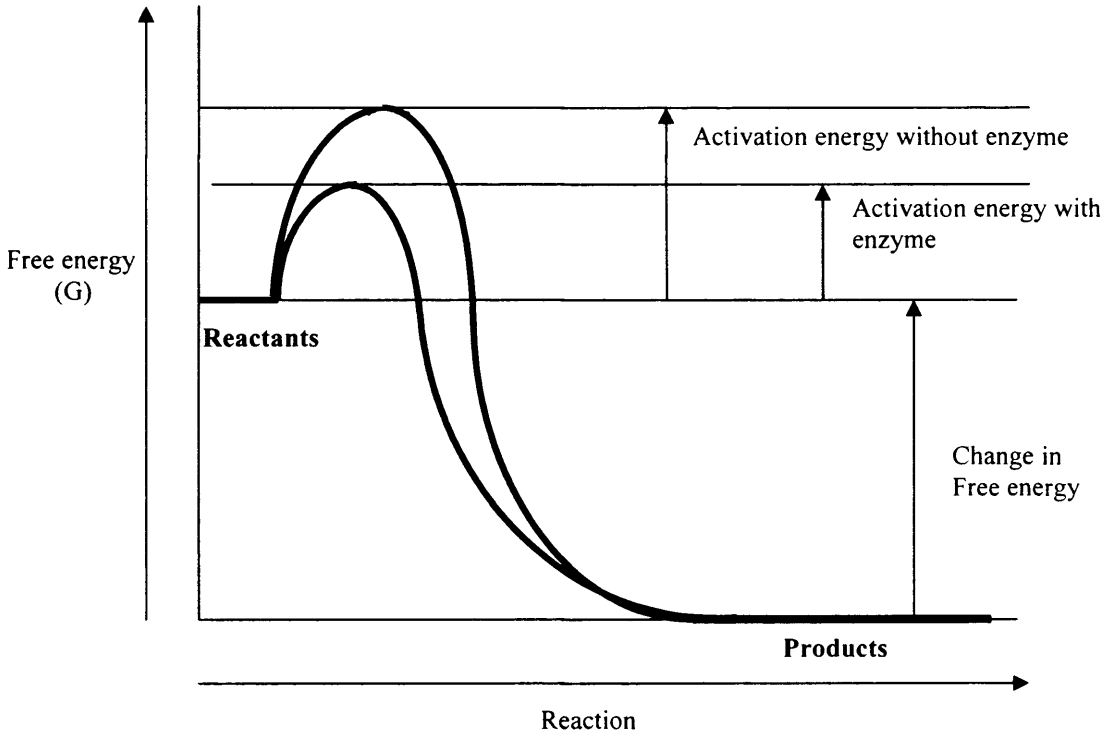


Figure 4. Enzyme activation

Enzymes are generally globular in structure and contain a unique three dimensional configuration as a result of the specific amino acid sequence. Within every enzyme there is a region known as the active site; the shape of this area is specific to the substrate which it binds to by covalent and hydrogen bonding, among others. The active site is an indentation of the enzyme's surface

established by the tertiary structure of the protein. In 1894, Emil Fischer[6] first postulated the specific action of an enzyme with a single substrate can be explained using a 'lock and key' model. The analogy proposed that the enzyme is a lock and that the substrate is the key. An important development, known as induced fit model, proposes that the enzyme undergoes a conformational change in shape in order to accommodate the substrate. Some active sites require the aid of a prosthetic group such as magnesium or zinc ions.

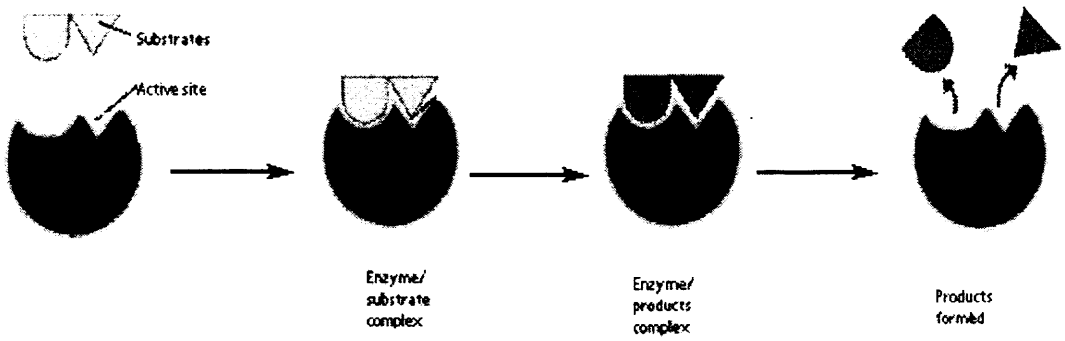


Figure 5. Depiction of Fischer's Lock-and-key model

Each enzyme has a unique three-dimensional structure which can be altered as a result of many factors. If the pH of the enzyme environment is altered then the bonds holding the enzyme together can change. Temperature is another factor that if increased can break the bonds (hydrogen bonds, hydrophobic interactions etc.) which hold the enzyme in a given conformation.

1. 6. 1 Enzyme kinetics

The study of enzyme kinetics was first described by Michaelis and Menten in 1913.[7] They both developed a quantitative theory of enzyme catalysis and kinetics. Their theory postulates first the enzyme (E) binds with the substrate (S) to form an enzyme-substrate complex (ES). The second step

postulated involves the enzyme-substrate breaking down to form a product (P) with regeneration of the free enzyme.

$$\mathbf{E + S} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \mathbf{ES}$$

$$\mathbf{ES} \underset{K_4}{\overset{K_3}{\rightleftharpoons}} \mathbf{E + P}$$

Michaelis-Menten equation

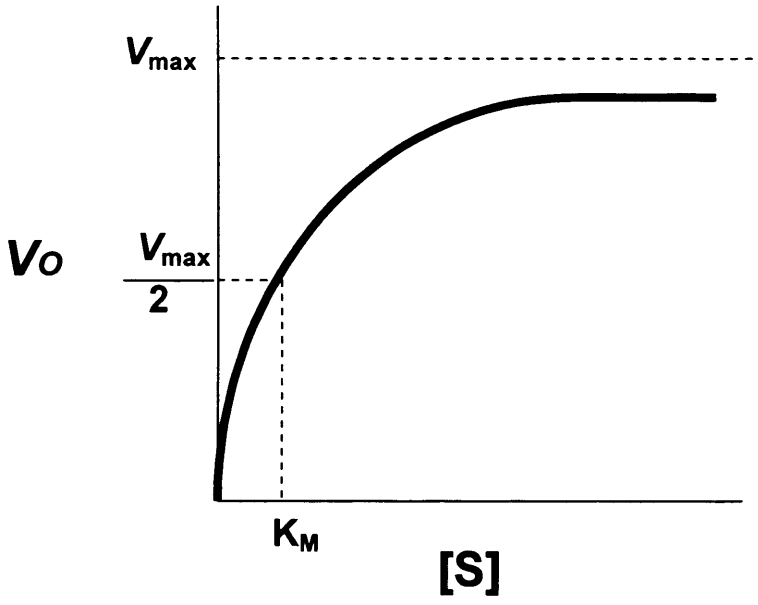


Figure 6. Michaelis-Menten kinetics: the effect of substrate on the initial rate

K_1, K_2, K_3 and K_4 are the rate constants of the reaction. The equations describe a reversible binding, although enzyme release and product formation is not always reversible. When the rate of enzyme substrate concentration is equal to the rate of breakdown a steady state is reached where [ES] is constant:

$$K_1([E_{free}]-[ES])\,[S] = K_2[ES] + K_3[ES]$$

Figure 7

This equation can be rearranged and expressed in terms of a K_M constant known as the Michaelis-Menten constant, which expresses the enzymes relationship to the substrate. The initial rate of a reaction is defined as V and is the measure of < 10% conversion of substrate into product. Once all the enzyme is converted into the enzyme substrate complex a maximum velocity rate is achieved, abbreviated as V_m. Michaelis-Menten derived an equation (i) to quantify the rate of reaction for the conversion of a one enzyme-one substrate reaction.

$$V = \frac{V_m [S]}{K_m + [S]} \quad \text{(i)}$$

The Michaelis-Menten equation

However, the Michaelis-Menten equation does not readily disclose the V_m when plotted onto a graph. In the Lineweaver-Burk representation[8] used the corresponding double reciprocal plot of the equation is used for the determination of an accurate value of V_m. When $1/V$ is plotted against $1/[S]$, a linear plot is obtained (Fig 8).

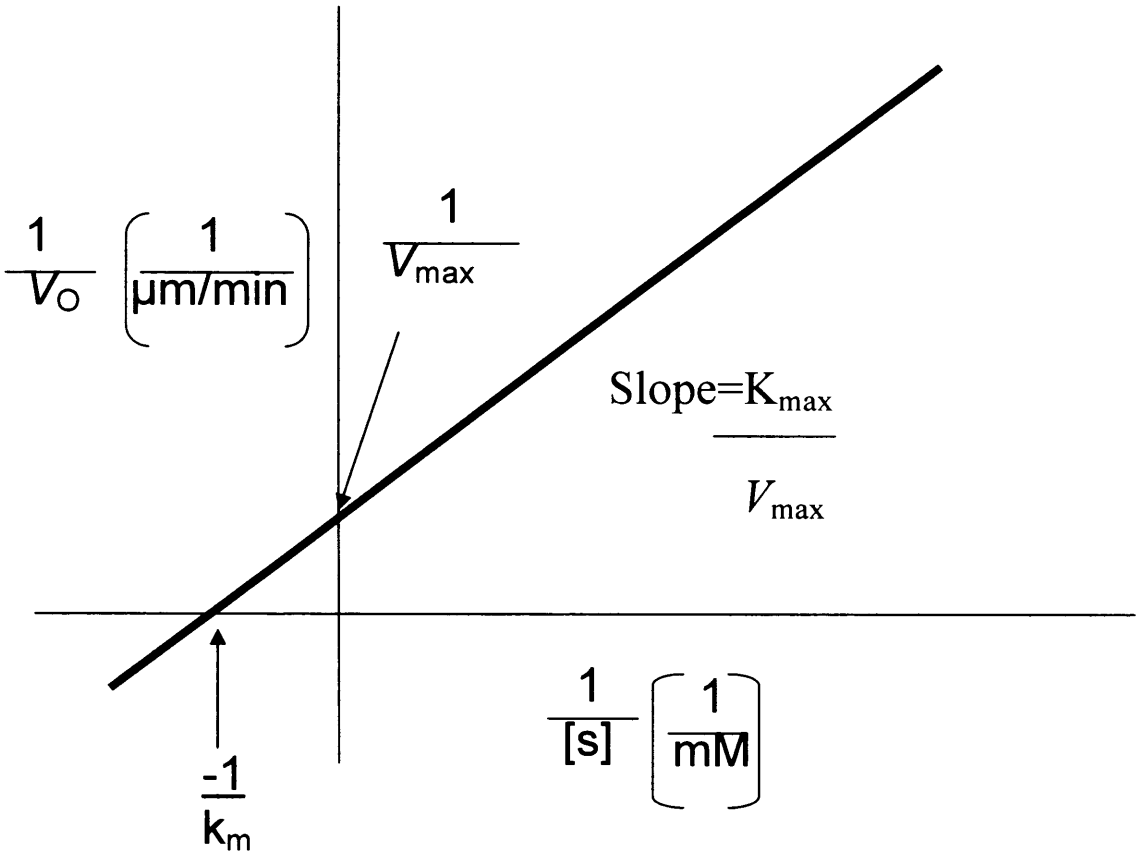


Figure 8. Lineweaver-Burk plot

Once $1/V$ is plotted against 1/[S], a linear relationship is obtained. The plot intercepts the y-axis at $1/V_m$ and extends onto the x-axis at $1/K_m$.

1. 6. 2 Enzyme Inhibition

Compounds known to inhibit enzymatic reactions are termed inhibitors. These inhibitors consist broadly of two types: known as reversible and irreversible. Reversible inhibition can be further subdivided into competitive, non-competitive and uncompetitive inhibition. Irreversible inhibition includes suicide and transition-state inhibition.

1. 6. 2. 1 Reversible inhibition

i) Competitive inhibition involves the substrate and the inhibitor competing for the same active site. Such inhibitors can exhibit a similar conformation to that of the substrate.

ii) Non-competitive inhibition includes inhibitors that are not competing for the active site but are able to bind to another location on the enzyme.

iii) Uncompetitive inhibition arises when an inhibitor is not competing with the enzyme or substrate but is in fact attacking the enzyme substrate complex.

1.6. 2. 2 Irreversible inhibition

Irreversible inhibitors bind to an enzyme and are not able to dissociate themselves from it. The inhibitor can usually form covalent bonds with the enzyme which makes it very stable. The inhibitor can act in two ways; firstly, it can destroy the active site or it can prevent the substrate from binding to the enzyme. *Suicide inhibition* is one form of irreversible inhibition. The enzyme would carry out its normal biotransformation with the inhibitor believing it to be the substrate but in fact the inhibitor forms a strong covalent bond which can deactivate the enzyme. *Transition state inhibitors* are inhibitors that can mimic the conformation of the active site when a substrate is bound to an enzyme. A well known transition state inhibitor is that of penicillin.

1.7 Telomerase

Telomerase is a reverse transcriptase enzyme and contains RNA as a template for telomeres. Telomeres are stretches of repetitive DNA structures which define the ends of chromosomes. The sequence of DNA is guanine-rich, consisting of $(TTAGGG)_n$ bases;[9] however, one strand is longer than the complimentary strand exposing one G base and is known as G-strand overhang. Telomeres can form a higher-order chromatin structure known as a T-loop which can hide the 3'-chromsome end during cellular activities, as shown in figure 9.[10] TRF1 and TRF2 are telomere binding proteins found around the T-loop which prevent telomere lengthening.

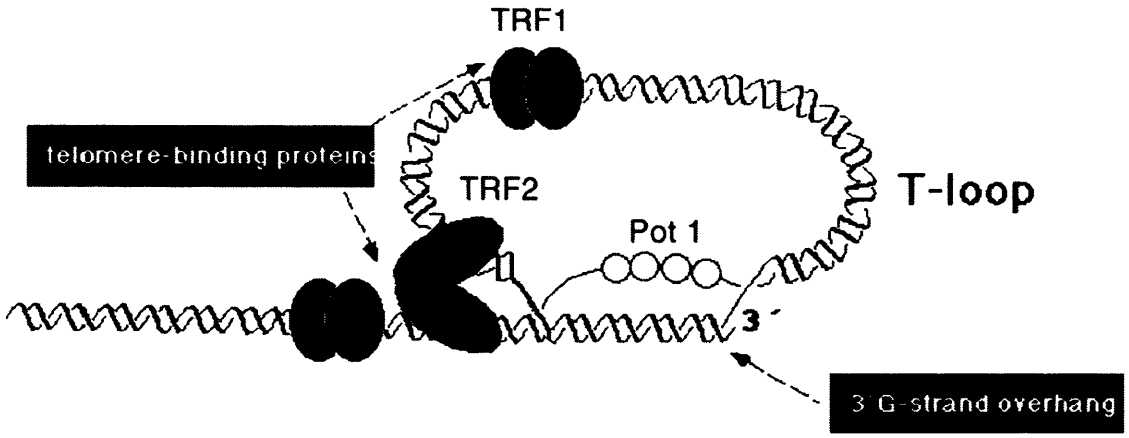


Figure 9.[10]

Telomerase is a specialised reverse transcriptase that synthesises telomeric repeats onto chromosomal ends and thus compensates for progressive telomere shortening caused by the end-replication problem.[11] Research into telomere biology has increased over the last decade, providing more understanding of its role in cancer and its prevention. Telomerase is an essential component of cellular immortalization and tumorigenesis.[12,13]

It is uncommon to find somatic cells which contain telomerase unless they are germ cells or stem cells; conversely, telomerase is shown to be activated in 85-90% tumour cells.[14,15] In most human somatic cells, telomeres shorten progressively by 50-200 nucleotides with each cell division. It is believed that

reduction in telomere length can lead to growth arrest. In old age, telomerase activity lessens and telomere shortening increases, which over the course of evolution has become one of the body's defences to cancer.[16] Anti-sense oligonucleotides and related compounds exhibit potent inhibition of telomerase in extremely low picomolar concentrations.[15]

From many viewpoints inhibition of telomerase is seen as an important target for a new anticancer treatment.

As mentioned, telomerase is a reverse transcriptase enzyme which consists of two major components comprising a RNA moiety (RTER) and a catalytic moiety subunit (hTERT) (Fig 10). These sites have been used as targets for telomerase inhibition. Many regulatory proteins associate themselves with the core, including hsp 90, p23 and TEP1.[17]

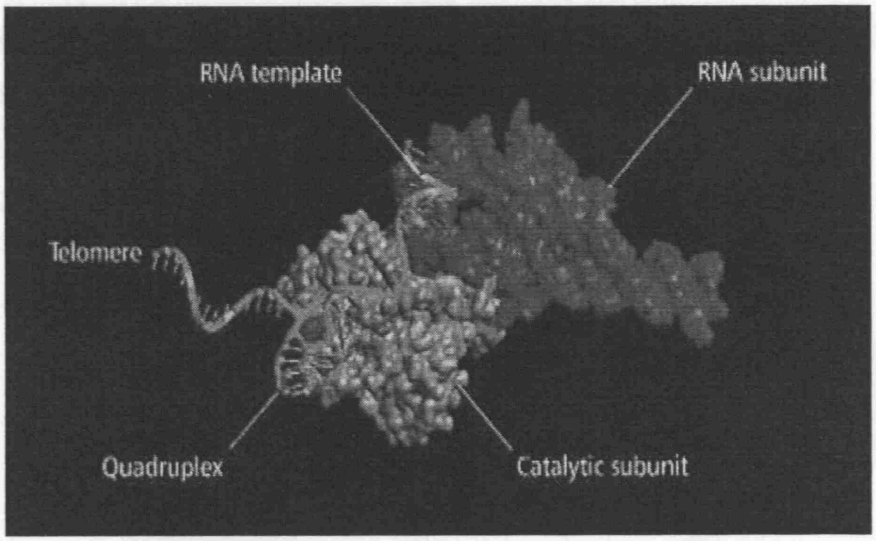


Figure 10. Structure of telomerase enzyme complex[1]

There has been much investigation into the direct targeting of the core telomerase components RTER and hTERT. In 1995 the first successful attempt to inhibit the enzyme directly was reported, results showing that telomeres of the treated cells had their nucleotides shortened. Kondo *et al* [18] carried out similar tests using the same vector on glioma cells showed the same results.

In 1997 a gene was discovered for the catalytic subunit of the telomerase enzyme which was later targeted by Zhang *et al*;[19] their results showed that telomeres had shortened, and that eventual apotosis of the cell occurred.

1. 7. 1 Biochemistry of Telomerase

The first hint of an activity capable of telomere synthesis was found by McClintock who found that specific maize tissues could repair themselves from broken chromosome ends; this was done by telomeres fusing the ends together.[20, 21] Then, 40 years after the discovery of telomere synthesis, Blackburn and co-workers cloned the first telomerase DNA found in *Tetrahymena thermophila*.[22]

Telomerase represents a truly unique enzyme that can synthesise telomeric DNA, one nucleotide at a time, in an apparently template-independent manner.[22] Greider *et al* [23] found that telomerase can elongate almost any G-rich, single-stranded DNA but the resulting sequence will always contain the newly added bases TTGGGG, as found in *tetrahymena* (fig 11).

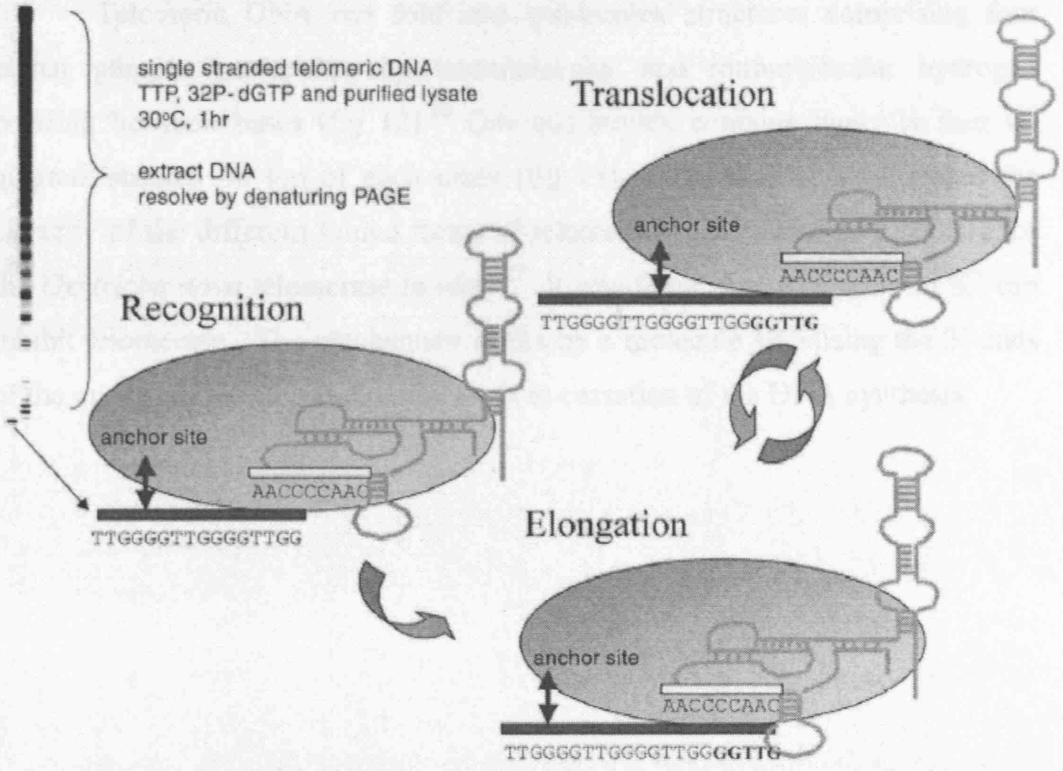


Figure 11 The telomerase elongation reaction.[24]

Greider proposed that the RNA component served as a template for telomere addition.[25] The reaction mechanism found for telomerase involves the replication of the telomerase RNA template to form a new telomeric DNA.

1. 7. 2 Telomere Targeting

Other targets of telomerase inhibition involve targeting the telomeres alone, the approach being taken in this project. Telomeres are comprised of chromatin and have specific binding protein sites on them.[17] Telomeres help to protect the chromosomes from recombination and fusion. At every cell division that takes place at least 100 bases of telomeric DNA are lost owing to DNA polymerase being unable to replicate the ends. However, in cancerous cells the telomere length is maintained and stabilized by the enzyme telomerase which catalyses the synthesis of TTAGGG repeats onto the 3'ends of the telomeres.

1. 7. 3 G- Quadruplexes

Telomeric DNA can fold into quadruplex structures comprising four planar guanine molecules, by intermolecular and intramolecular hydrogen bonding between bases (fig 12).[26] One quadruplex contains typically four G-quartets stacked on top of each other (fig 13). Zahler *et al* investigated the capacity of the different folded forms of telomeric DNA to serve as primers for the *Oxytricha nova* telomerase *in vitro*.[27] It was found that formation of K^+ can inhibit telomerase. The mechanism works by a molecule stabilising the 3' ends of the quadruplex which eventually leads to cessation of the DNA synthesis.

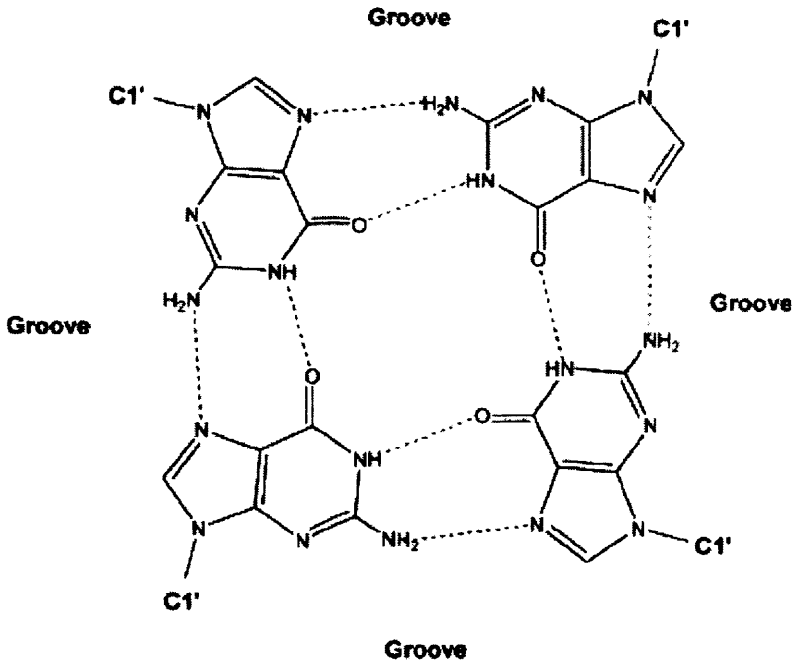


Figure 12[27]

A property of G-rich, single stranded DNA is that it can fold into a four-stranded structure assembled around a core stack of guanines arranged in almost-planar hydrogen-bonded tetrads.[28] Molecules which are able to carry out this mechanism are planar, electron deficient ring systems usually bearing acyclic substituents (fig 14). Molecules that are planar tend to be selective at stabilizing G-quadruplexes because they can fit through the folds easily and interact with the bases. Small molecules that stabilise or promote the formation of quadruplexes also show inhibitory activity.[29]

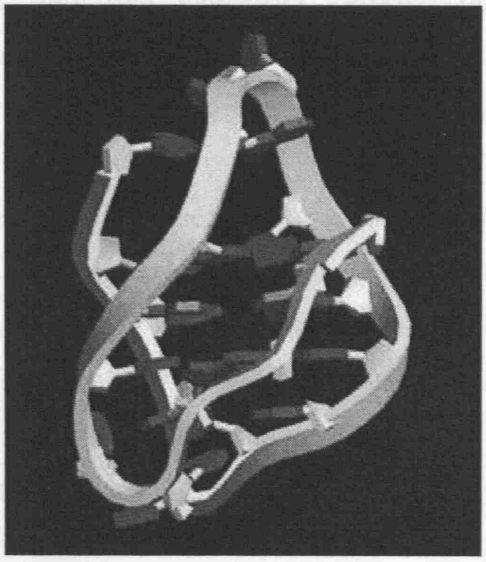


Figure 13. Model showing the intramolecular bonding occurring in human quadruplex[1]

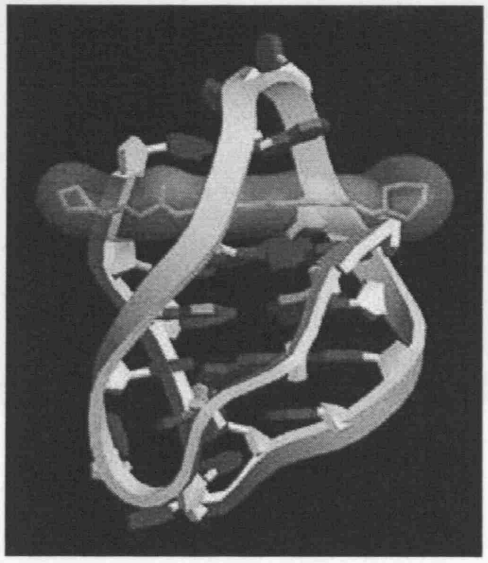


Figure 14. Model showing how a planar and electron deficient molecule fits between the G-quadruplex to inhibit the enzyme telomerase.[1]

Only a few small molecules have been identified as quadruplex ligands; they are subdivided into two categories: groove binders and aromatic chromophores. Examples of aromatic chromophores include porphyrins that contain a cation, anthraquinones, and several tri-and tetra-cyclic ring systems.

Porphyrins with cations attached to them are potent inhibitors, primarily because of the planar chromophore structure which has the ability to fit into the grooves of the quadruplex (fig. 15). Secondly, the cations bind to the negatively charged phosphate back bone of DNA to stabilise the G-quadruplex followed by telomerase inhibition. Such compounds have provided moderate telomerase inhibition with IC_{50} values of 1-25 μM.[28]

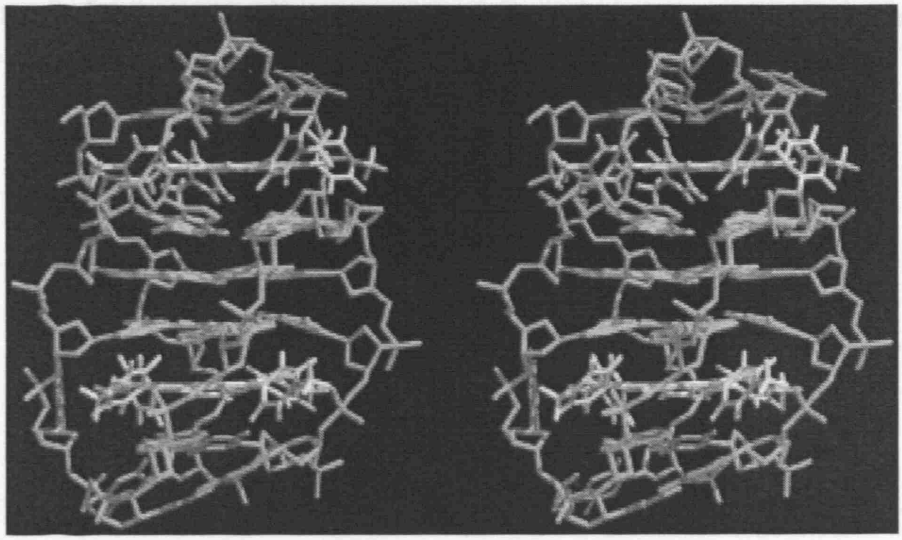


Figure 15[28]

1.7.4 Biological studies on telomerase inhibitors

In the TRAP assay, telomerase activity can be measured *in vitro* by a primer extension assay in which telomerase synthesises telomeric repeats onto oligonucleotide primers.[30] Hence, telomerase synthesises extension products are used in PCR amplification.[31] In most biological assays for telomerase inhibition a TRAP assay is used.

Many groups have synthesised compounds that inhibit telomerase by targeting G-quadruplex DNA. A series of metalloporphyrins was prepared by Maraval and co-workers;[32] the porphyrins were metallated with either manganese or nickel. Results showed that the complexes were able to inhibit the telomerase enzyme with IC_{50} values in the micromolar range.[33] Neidle and co-workers[34] have synthesised telomerase inhibitors that are symmetrical 2,6-disubstituted aminoalkylamido anthraquinones (fig 16) and which possessed IC_{50} values < 10 μM .

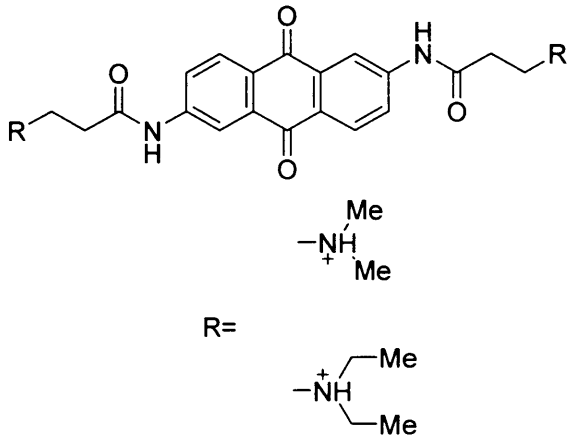


Figure 16. 2,6-Disubstituted aminoalkylamido anthraquinone telomerase inhibitors[33]

Sasaki *et al* found a successful inhibitor of a protein which activates the DNA replication in *E.coli.*[15]Further tests of these compounds have shown that these inhibitors can also suppress telomerase by up to 90%. The structures of these inhibitors are based on the acetoxy-substituted bi-indole template shown in (figure 17). SAR data shows that a carboxylic acid group and a spacer between the bi-indole of exactly not more or less than 14 methylene groups provide successful inhibitors.

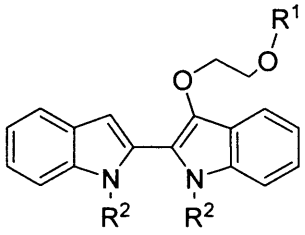
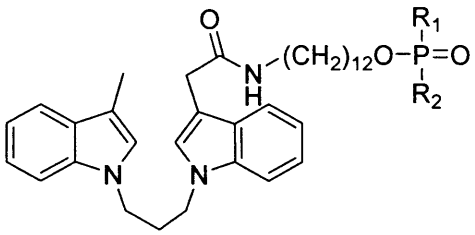


Figure 17

	Compounds		Inhibition (%)
No.	R^1	R^2	
1	$NH(CH_2)_{11}COOH$	H	68
2	$NH(CH_2)_{11}COOCH_3$	H	8
3	OH	H	9
4	CH_3	H	1
5	$NH(CH_2)_2COOH$	H	0
6	$NH(CH_2)_5COOH$	H	0
7	$NH(CH_2)_5CONHCH_2COOH$	H	13
8	$NH(CH_2)_{11}CONHCH_2COOH$	H	36
9	$NH(CH_2)_4CONH(CH_2)_5COOH$	H	56
10	$NH(CH_2)_{11}CONH(CH_2)_{11}COOH$	H	0
11	$NH(CH_2)_{11}COOH$	CH_3	90
12	$NH(CH_2)_{11}COOCH_3$	CH_3	0
13	$NH(CH_2)_{11}COOH$	CH_2Ph	73

Table 1

Results (Table 1) showed that a terminus carboxyl group provided the best result; a phosphate group (fig 18) was also tested as it possesses the same anionic charge at physiological pH. SAR data (table 2) showed very low IC_{50} values of 2.5 μM, making it the most potent telomerase inhibitor in this series.



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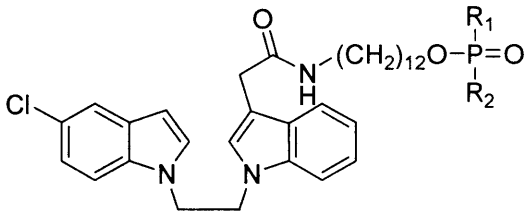


15

Figure 18

	Compounds			Inhibition (%)
No.	Type	R^1	R^2	
1	**14**	OCH_2CH_2CN	OC_6H_4-Cl-*m*	20
2	**15**	OCH_2CH_2CN	OC_6H_4-Cl-*m*	6
3	**14**	$OH{\cdot}Et_3N$	OC_6H_4-Cl-*m*	100
4	**15**	$OH{\cdot}Et_3N$	OCH_2CH_2CN	41
5	**15**	$OH{\cdot}Et_3N$	OC_6H_4-Cl-*m*	100
6	**15**	$OH{\cdot}Et_3N$	OC_6H_5	100
7	**15**	$OH{\cdot}Et_3N$	O-cyclohexyl	100

Table 2. SAR studies of novel telomerase inhibitors based on phosphate derivatives.

Yin *et al*[26] has reported a novel telomerase inhibitor which can successfully stabilise the G-quadruplex. These compounds are linear structures made up of triethylene tetraamine (TETA) (fig 19). The compound possesses the desired properties for G- quadruplex stabilisation; which are planar electron deficient chromophore, basic side chains, similar to that of anthraquinones.

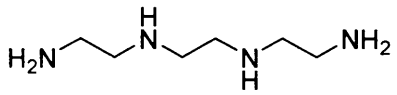


Figure 19

Biological results showed that TETA was very potent obtaining an IC_{50} value of 7.8 μM. The precise mechanism of contact with G quadruplex remains unclear, however, it is possible to assume the compound interacts by inter and intramolecular and hydrogen bonds.

Maraval *et al* [32] synthesised a tetracationic porphyrin, known as *meso*-tetrakis(4-*N*-methylpyridinyl)porphyrin (fig 20) which possesses properties that make it bind well with the G-quadruplex.

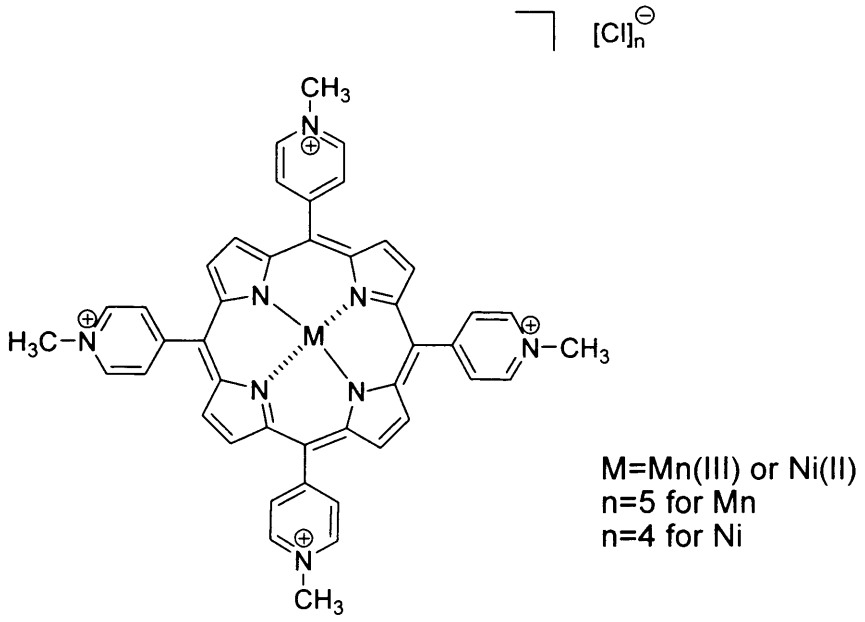
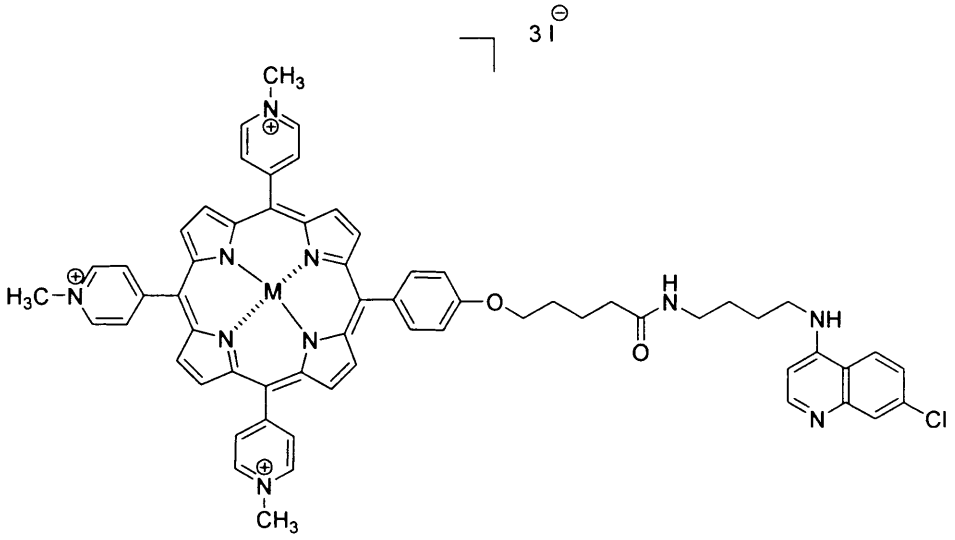


Figure 20

The metalloporphyrins were prepared from a condensation reaction involving pyrrole and 4-methylpyridinylbenzaldehyde.[37]

CD and UV thermal melting studies have shown that telomerase inhibition takes place by π-stacking between the G-quartet, owing to its planar, electron deficient structure. Maraval *et al.*[32] showed that manganese metal ions can undergo an oxidation reaction involving $KHSO_5$ into an oxomanganese species; which can oxidise guanine residues in DNA. *In vivo* oxygen in a molecular form is available to oxidise manganese ion in the porphyrin structure.

Optimal inhibition of telomerase was carried out by changing the pyridinyl groups into aminoquinoline groups, **16** (Fig 21). IC_{50} values of around 5-10 μM were obtained for these compounds.



16

Figure 21

Dong-Fang Shi *et al* [28] have researched into telomerase inhibitors focussing on G-quadruplexes. The compounds they synthesised were of a porphyrin type structure TMPyP4 (fig 22) similar to that of Maraval *et al.*; however, different functional groups were attached in order to accumulate as much SAR data.

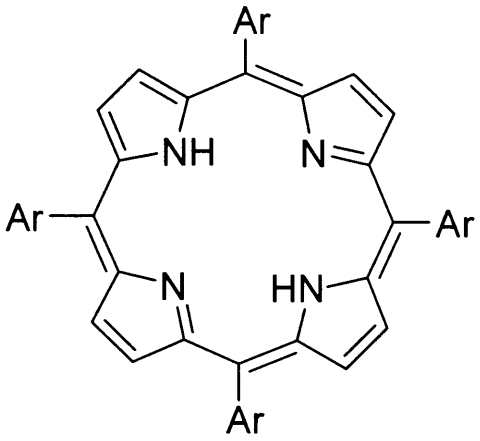


Figure 22 Structure of TMPyP4

From their results they concluded that a cationic porphyrin showed enhanced potency which worked at IC_{50} values at 5-25 μM. It is found that using free porphyrins, photocleavage of DNA can arise which is a dangerous consequence for *in vivo* studies. Using Cu(II) can provide an unhindered porphyrin square planar conformation, providing 75% inhibition without causing any photocleavage. Mn (III) and Mg (II) both form octahedral complexes that help prevent the stacking inside the G-quartet, hence, providing low telomerase inhibition values summarised in Table 3.

Porphyrin	Metal ion	Geometry	% Inhibition (25μM)
TMPyP4	H2		88
	Zn (II)	Py	88
	Co (II)		83
	Fe (III)	oh	63
	Ni (II)	sq pl-oh	42
	Mn (III)	oh	37
	Cu (II)	sq pl	75
	Mg (II)	oh	42
	Pt (II)	sq pl	69
	Pd (II)	sq pl	41

Table 3.

For effective telomerase inhibition, substituents containing a positive charge would be desirable. The reason for this is based on the nature of DNA, which has a back bone of phosphate anions; accordingly, ligands can interact better with DNA if a positive charge is present. SAR results show that with more charged substituents present on the porphyrin structure, the telomerase inhibition can increase to 25 μM Table 4.

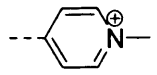
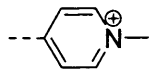
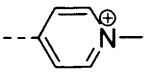
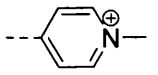
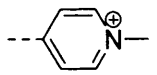
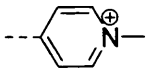
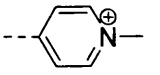
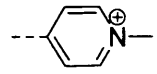
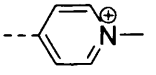
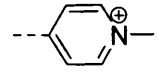
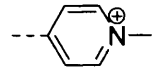
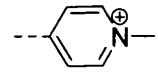
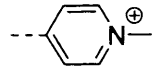
Porphyrin	5-Ar	10-Ar	15-Ar	20-Ar	inhibition (%)
TMPyP4					62
				Ph	15
		Ph		Ph	30
			Ph	Ph	30
		Ph	Ph	Ph	30
		4-Tol	4-Tol	4-Tol	31
	Ph	Ph	Ph	Ph	31

Table 4.

Positively charged *N*-position substituents are essential for telomerase inhibition. Such alkyl groups used in the SAR studies have included methyl, ethyl and hydroxyethyl groups which have shown some inhibition; however, with increasing chain length the inhibition is decreased. The substituents providing the highest degree of inhibition are that of the *N*-methylpyridinium series. If the substituent contains a Zwitterion, no net charge is present to interact with the DNA, resulting in a decrease in telomerase inhibition as shown in Table 5.

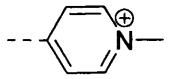
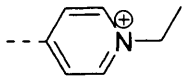
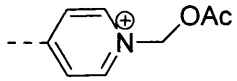
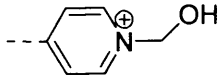
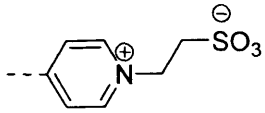
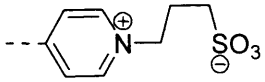
Porphyrin	*meso*-Ar	inhibition (%)
TMPyP4		67
		55
		15
		55
		0
		14

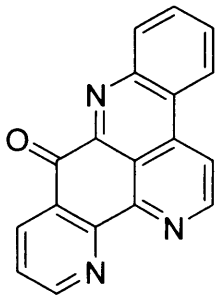
Table 5

1. 8 Natural products

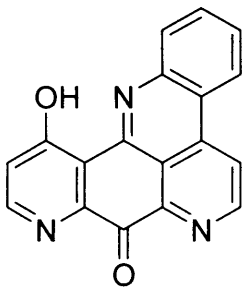
Many natural products can act as anticancer agents, and many research groups are searching for new compounds from marine sponges for their neoplastic effects. Unsurprisingly, numerous natural products are in clinical trials for the treatment of cancer.

1. 8. 1 Ascididemin and Meridine

Ascididemin was isolated by Kobayashi. *et al.*[35] In 1988 from the *Okmauratumal didemnum*. Meridine was isolated by Schmitz *et al.*[36] In 1991 from *Amphicarpa meridiana;* these natural products belong to the pyridoacridine family which are found in marine sponges. The main structural differences (fig. 23) are that Ascididemin has a pyridine ring attached to the acridine skeleton, whereas Meridine contains fused phenolic type ring.



Ascididemin



Meridine

Figure 23

These natural products have been shown to behave as telomerase inhibitors by stabilising the G-quartet. Using the TRAP assay to measure the degree of telomerase inhibition it was found that Ascididemin and Meridine had IC_{50} values of 87 μM and 11 μM respectively.

1. 8. 2 Dictyodendrins 17-20

There are many compounds that can act as telomerase inhibitors.

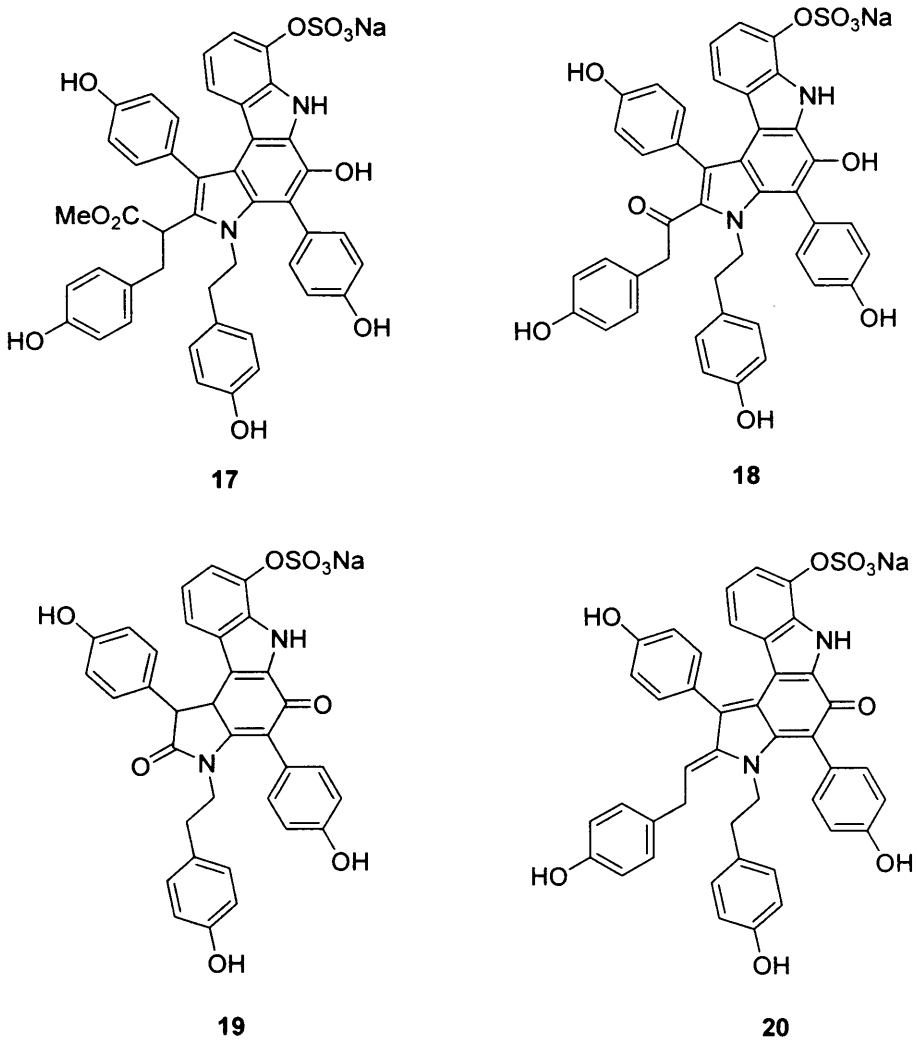


Figure 24

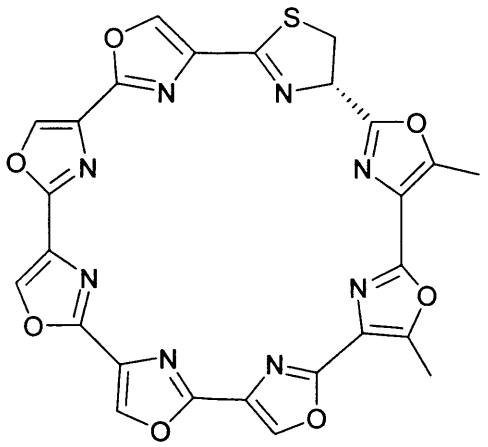
Such examples include Korean mistletoe, tea catechins and compounds from the Japanese marine sponge *dictyodendrilla verongiform.*[37] These compounds are thought to act as telomerase inhibitors via the G-quadruplex which causes telomerase shortening.

Dictyodendrins **17-20** showed 100% inhibition of telomerase at a concentration of 50 μg/ mL scale.[37] These natural products were the first known

telomerase inhibitors and researchers are synthesising analogues of them to enhance potency.

1. 8. 3 Telomestatin

A natural product known as telomestatin (**21**) (Fig 25) can target G-quadruplexes and is shown to be very potent with activity at 5 nM. In 2001 Shin-ya *et al.*[38] successfully isolated telomestatin from *steptomyces anulatus* 3533-SV4. Telomestatin can specifically inhibit telomerase without affecting the DNA polymerase or reverse transcriptase found in the enzyme, making it a very selective compound.



21

Figure 25. Structure of the natural product telomestatin

Telomestatin was successfully isolated by first cultivating *Steptomyces anulatus*[39] 3533-SV4 in 2% glycerol, 1.0% polypepton and 0.4% $CaCO_3$ for three days in a fermentor. The mycelium was then collected after centrifugation. The organism was then extracted into acetone, which was evaporated to dryness and the residue was partitioned between ethyl acetate and water. The organic layer was isolated, dried and purified via column chromatography (methanol and chloroform). Chemical analysis was carried out on the purified compound using FAB-MS which found a molecular formula of $C_{26}H_{14}N_8O_7S$ was $(M+H)^+$, *m/z* 583.0790 (calcd 583.0784). Further spectral data including ^1H NMR and ^{13}C

NMR were obtained for the product. The ^{1}H NMR spectrum showed 5 aromatic signals δ_H 8.12, 8.13, 8.24 and 8.34 which were connected to carbon signals δ_C 137.5-141.2 in the HMQC spectrum. The long range coupling involving δ_C 130.4- 136.7 and δ_C 156.2-157.3 quaternary carbons established the presence of the linked polyoxazole system. Two methyl groups located on the oxazoles were found at δ_H 2.55 and 2.65 further confirmed the structure proposed. The thiazoline ring showed two peaks at δ_H 3.49 and 3.93. A simulated annealing docking approach was carried out on telomestatin to determine the binding interactions with the G-quadruplex of DNA. The docking approach showed that two molecules of telomestatin bind to the G-quartet in the intramolecular orientation shown in Fig. 26.

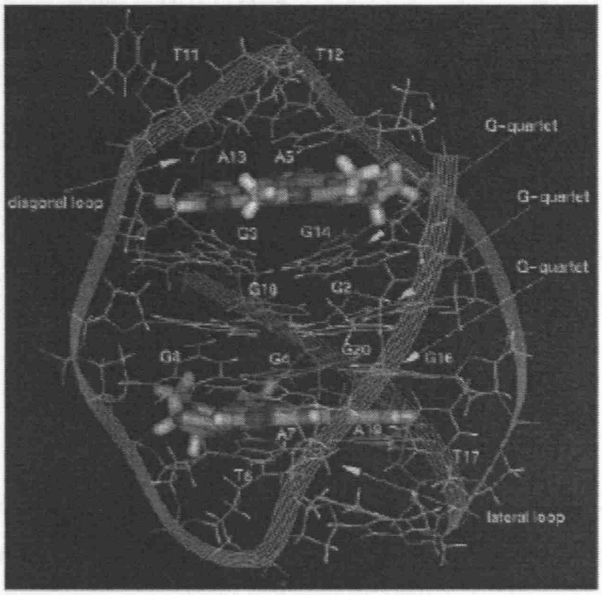


Figure 26[39]

Such natural products with telomerase inhibitory activity may be relatively non-cytotoxic and hence as potential of anticancer agents. Minhas *et al*[40] has reported the synthesis of a series of macrocycles (Fig 27) which contain a 24-membered ring similar to that present in telomestatin.

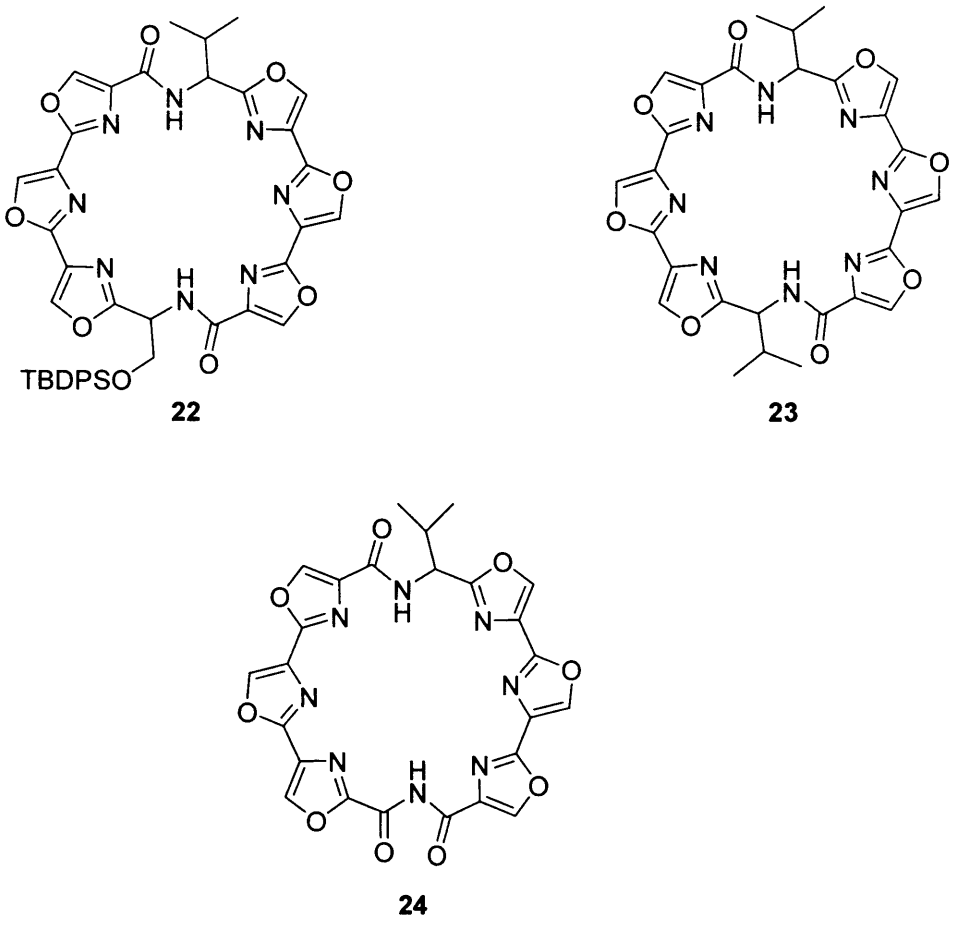


Figure 27. Polyoxazoles related to telomestatin[40]

Macrocycles **22-24** were evaluated for G-quadruplex binding and inhibition. UV studies were carried out on the macrocycles with G-quadruplex DNA being the function of temperature. According to the UV results there was no change in the melting transition temperature when the macrocycles were tested. Macrocycle **22** showed no change in the melting transition temperature even with d $(TTAGGG)_4$, indicating that no binding was taking place with G-quadruplex DNA. Macrocycles **23** and **24** exhibited an increase in the melting transition temperature of 73.5 °C, 62.5 °C respectively, showing that some binding was taking place. The macrocycles **23** and **24** were tested for inhibition of human lymohoblastoma RPMI 8402 cells, IC_{50} values obtained being 0.21 and 0.8 μM repectively.

1. 9 Oxazoles

1.9.1 Introduction

Oxazoles are numbered around the ring starting from the oxygen ring (figure 28).

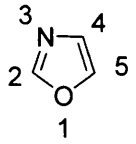
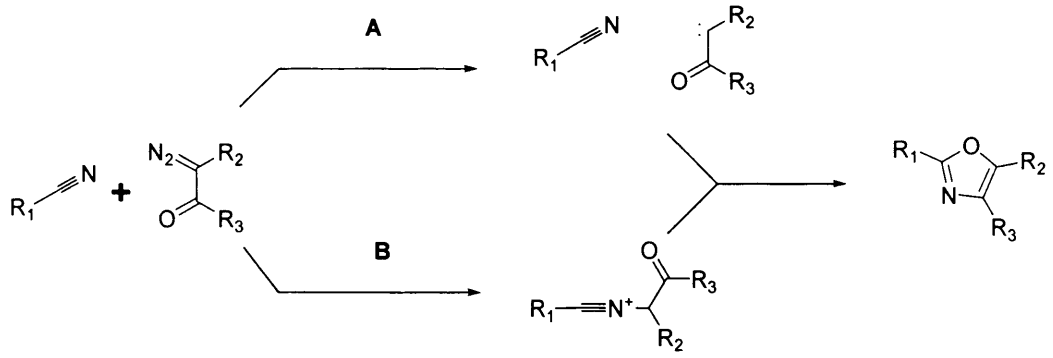


Figure 28

The proton acidities decrease in the order: 2 >5 >4, owing to the electron-withdrawing effect caused by the oxygen and nitrogen atoms. Oxazoles exhibit characteristic resonances from 7.0 to 8.0 ppm in proton NMR spectra and between 128 and 153 ppm in ^{13}C NMR spectra. These chemical shifts depend somewhat on which substituents are present at the different positions.

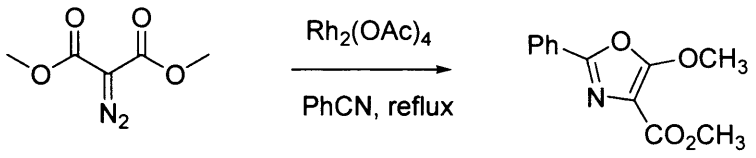
1. 9. 2 Synthesis of 1, 2, 4-trisubstituted oxazoles from nitriles

The synthesis involves the reaction of diazocarbonyl compounds with nitriles under thermal, photochemical, Lewis acid or metal catalysed conditions.[41] The reaction proceeds via a 1,3-dipolar cycloaddition of the carbonylcarbene to the nitrile (path A) or formation and subsequent 1,5-cyclisation of a nitrile ylide (path B).[41]



Scheme 2

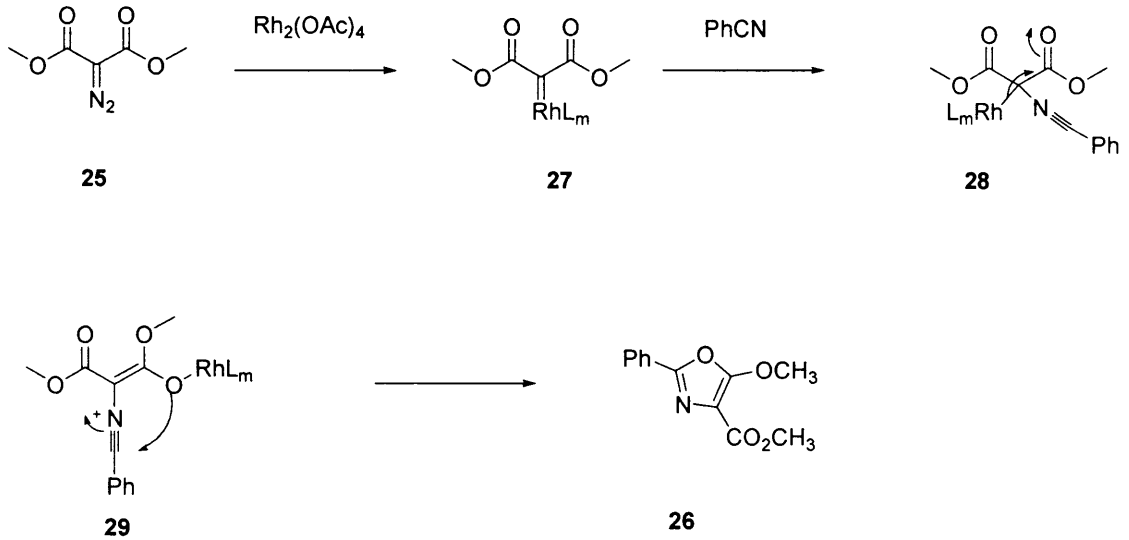
Helquist *et al.*[42] investigated metal-catalysed reactions involving rhodium with diazocarbonyl compounds and nitriles. Heliquist tested metal salts which included $Rh_2(NHAc)_4$, $Rh_2(O_2CC_3H_7)_4$ but chose rhodium(II) acetate, $Rh_2(OAc)_4$, as it provided the highest yield of the desired product.[42] The catalyst was reacted with dimethyl diazomalonate with benzonitrile which subsequently gave oxazole **26** (scheme 3).



25 **26**

Scheme 3

Helquist proposed a mechanism for the reaction (scheme 4). The diazo compound **25** reacts with the rhodium catalyst to generate a carbene complex **27** which is subject to nucleophilic attack at the electrophilic carbon centre found on the nitrile. A nitrilium species **29** then undergoes internal attack by an enolate oxygen to give the observed oxazole product **26**.[42]

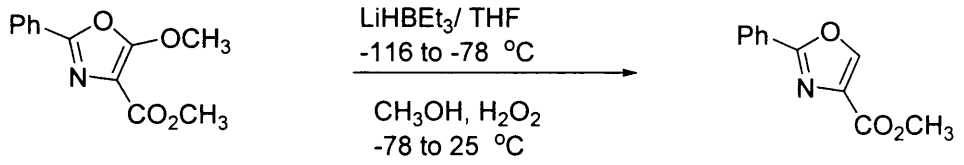


25 **27** **28**

29 **26**

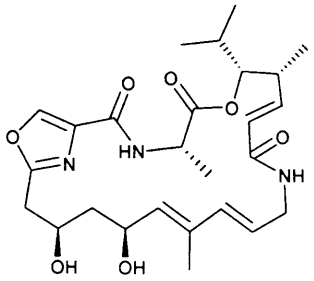
Scheme 4

The 5-methoxy group can be removed to leave a 2,4-disubstituted oxazole (Scheme 5), by reduction using $LiB(Et)_3H$ (Aldrich Super Hydride®) which acts as the reducing agent. Heliquist did this because several naturally occurring compounds bear these 2,4-disubstituted oxazoles, *e.g.* Mandumycin II, telomestatin (Fig. 29).



26

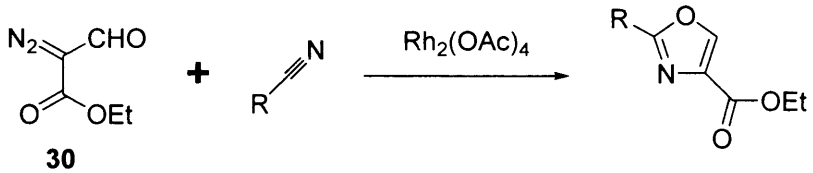
Scheme 5



Mandumycin II

Figure 29

Helquist *et al*[42] went on further to improve this method by eliminating the reduction step, by using diazoaldehyde ester **30** with nitriles (scheme 6).

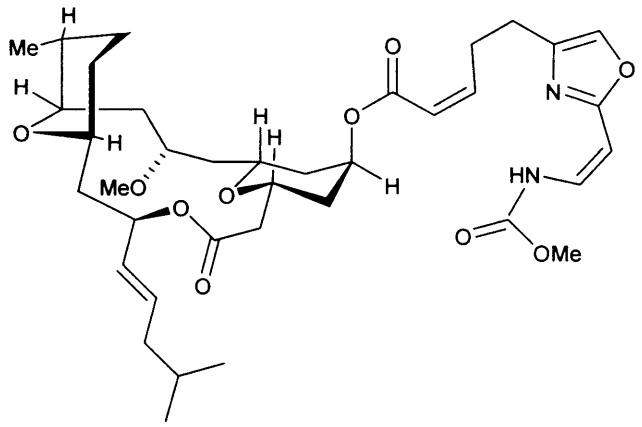


30

Scheme 6

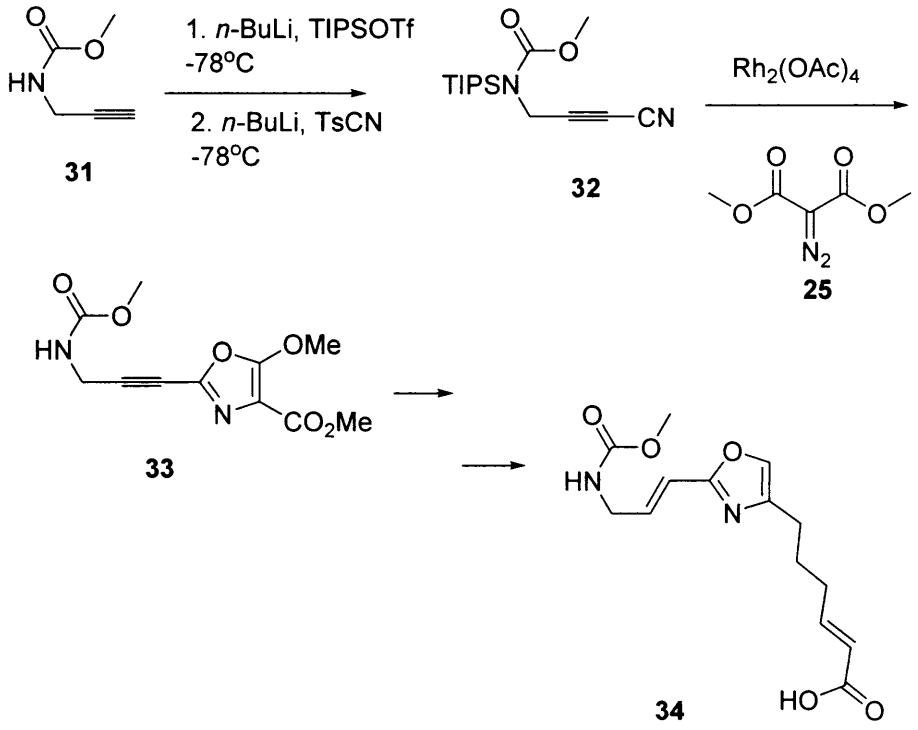
The diazoaldehyde ester was readily available by a Vilsmeier-Haack formylation.[42] For the oxazole formation the authors investigated a number of catalysts including $Rh_2(OAc)_4$, $BF_3.Et_2O$, $Cu(OTf)_2$ and $Pd(OAc)_4$, but only $Rh_2(OAc)_4$ was found to be effective. The optimum conditions used excess nitrile as the solvent and a temperature in the range of 65- 95 °C (scheme 6) but provided only low yields (18-45 %).

Kozmin *et al*[43] used the metal catalysed reaction in their synthesis of the natural product Leucascandrolide A[43] (Figure 30). The 2,4-disubstituted oxazole side chain was prepared by the reaction of a nitrile with diazomalonate in the presence of $Rh_2(OAc)_4$. The reaction was followed by protodesilylation to give the oxazole **33** (scheme 7). Further reactions were carried out to furnish the desired oxazole side chain **34**.



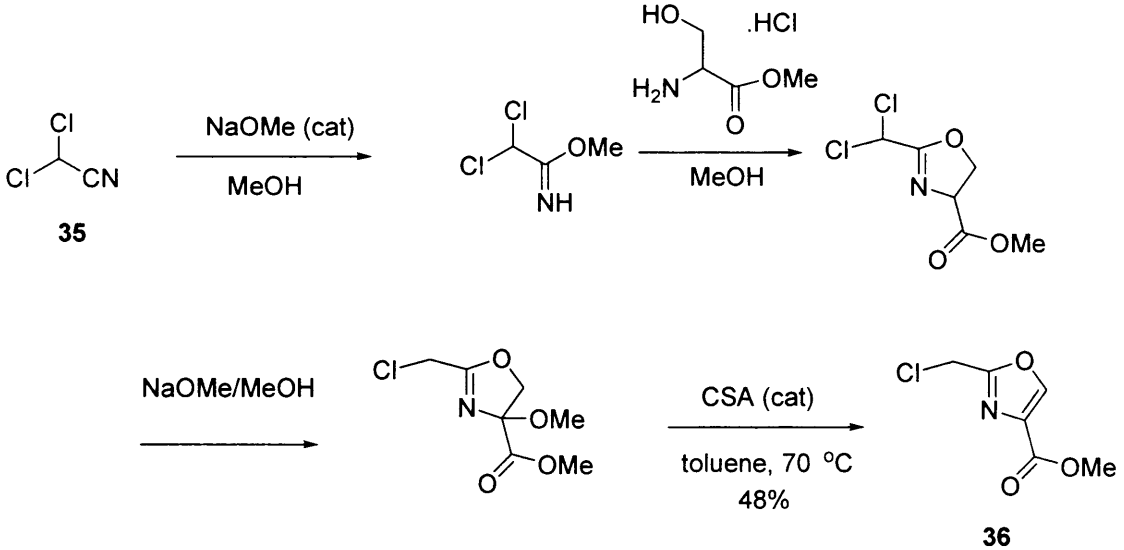
Leucascandrolide A

Figure 30



Scheme 7

Hermitage and co-workers[44] reported the synthesis of the 2,4-disubstituted oxazole **36**, from dichloroacetonitrile **35** (scheme 8). The reaction proceeds readily, to give the oxazole in a satisfactory yield of 48%. The reagents are readily available and are environmentally acceptable for an industrial scale synthesis.



Scheme 8

1. 9. 3 2,4-Disubstituted oxazoles synthesised from amides

Part of the 2,4-linked polyoxazole assembly present in telomestatin is also found in other natural products. A cyclopeptide known as YM-21631[45] (Fig. 31), isolated from *streptomyces nobilis,* shares similar structural and biological properties to that of telomestatin. The total synthesis of the natural product was accomplished by Pattenden *et al.*[43] The synthesis was carried out by preparing a tris-oxazole ring system derived from serine.

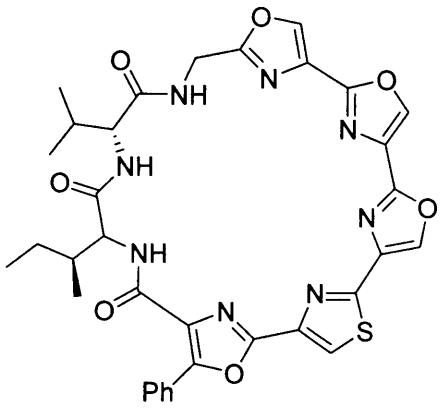
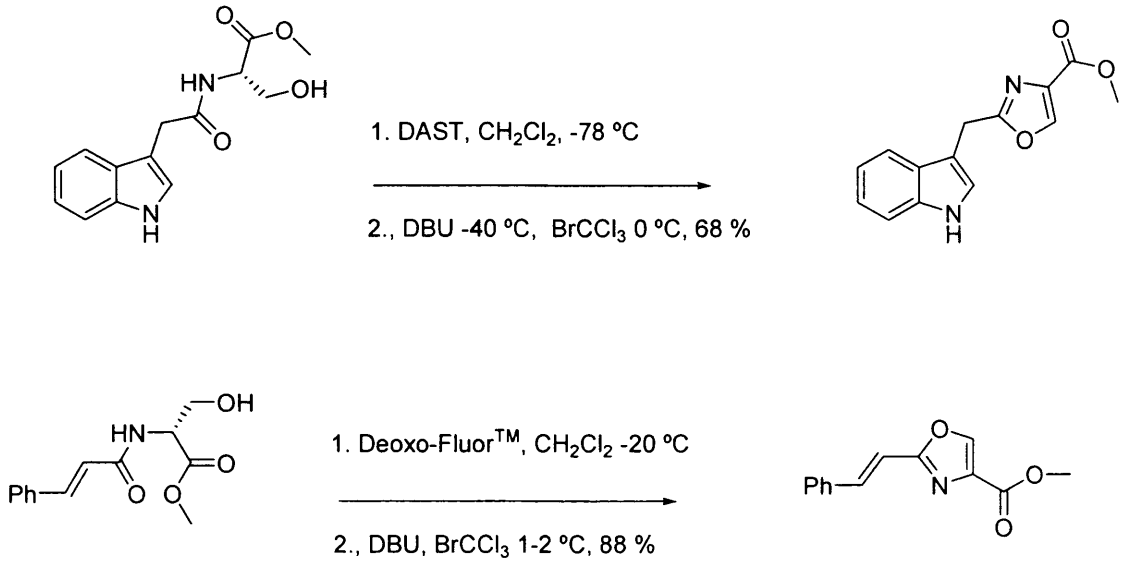


Figure 31. Cyclopeptide YM-21631

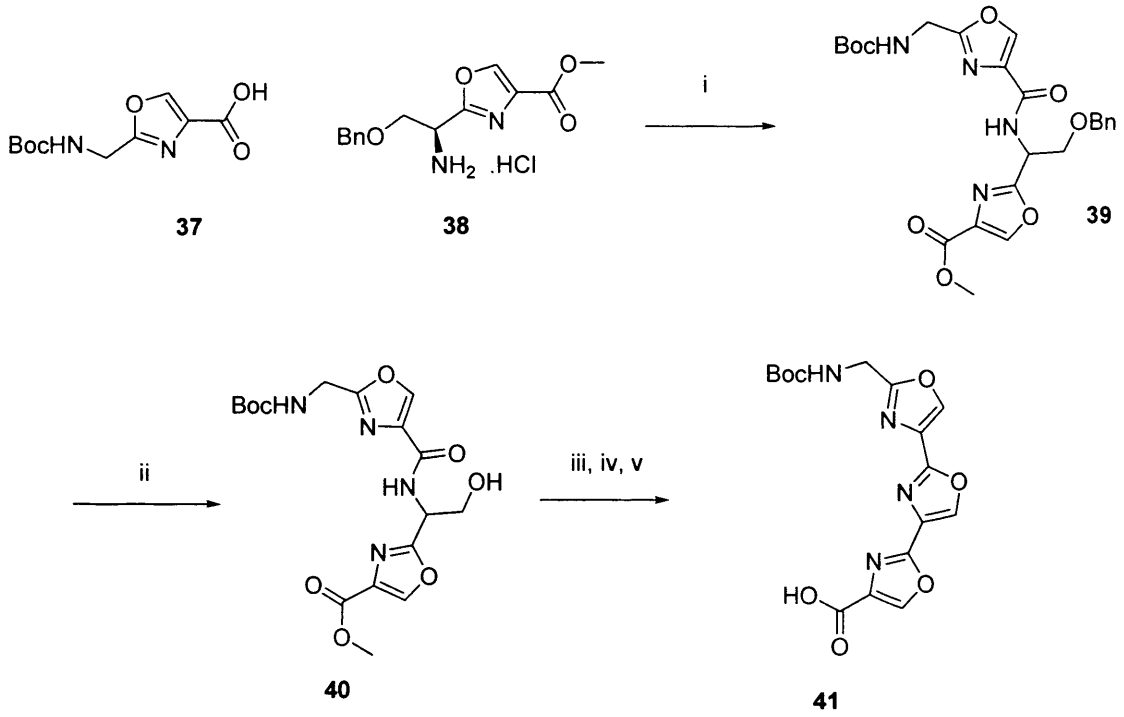
A procedure discovered by Wipf[46] and Williams[47] to synthesise an oxazole in one pot using diethylaminosulfur trifluoride (DAST), 1,8-diazabicyclo[5.4.0]undecene (DBU), bromotrichloromethane ($BrCCl_3$) and reacting these reagents with an amido alcohol. Wipf *et al.* also showed that a one-pot reaction to obtain an oxazole from an amido alcohol succeeded using bis (2-methoxyethyl) aminosulfur trifluoride (Deoxo-Fluor)^{TM}, DBU and $BrCCl_3$. DeoxoFluor^{TM} is found to be an alternative to DAST and preferable owing to its thermal stability. Unlike DAST which requires -78 °C to cyclise the amino alcohol (as above), Deoxo Fluor^{TM} requires only -40 °C (scheme 9). However, DAST provides better yields for serine-derived oxazoles, in comparison to Deoxo-Fluor^{TM}, which gives higher yields on theronine-derived compounds.



Scheme 9. Reagents for oxazole formation

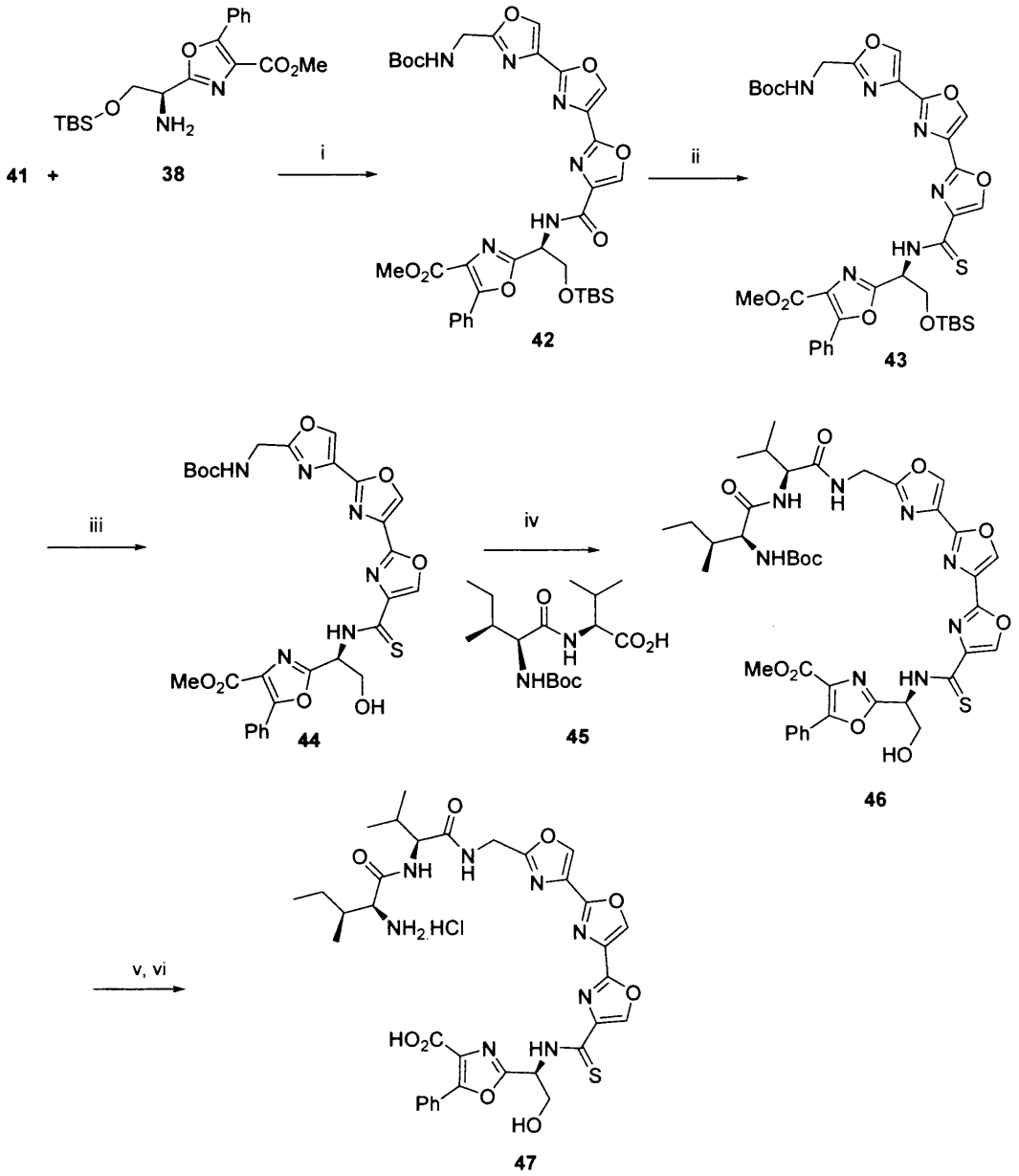
The 2,4-disubstituted oxazoles are linked to form a consecutive trisoxazole system. Scheme 10 below outlines the route taken. Of two oxazoles, one contained a terminal acid group and the other an amine. These two compounds were coupled together using EDCI, HOBT and NMM, giving amide **39** in 56% yield. The benzyl group was then deprotected by hydrogenolysis over palladium hydroxide to give a free hydroxyl group. The alcohol group had been previously protected in order to avoid elimination of water, a major side-reaction.

The Wipf procedure was used in the synthesis of YM-21631 (step iii scheme 10). The overall yield using the DAST, DBU and $BrCCl_3$ method was 57%, which is reasonable for the two-step process.



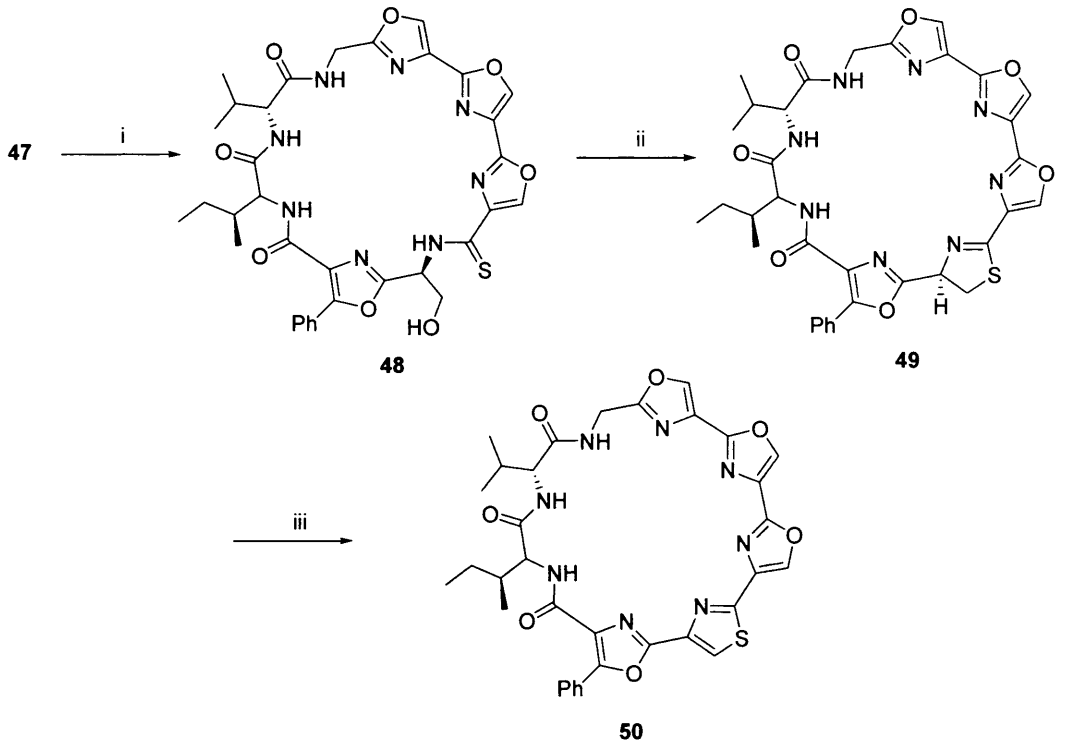
Reagents and conditions: i, EDC, HOBt, NMM, CH_2Cl_2, 0 °C, 24 h, 56 %; ii, H_2, 20% $Pd(OH)_2/C$, MeOH-THF (2:1), rt, 81 %; iii, DAST, CH_2Cl_2, -78 °C, 1.5 h; iv, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 24 h, 57 %; v, NaOH, THF, H_2O, rt, 24 h, 88%.

Scheme 10



Reagents and conditions: i, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to rt, 24 h, 87 %; ii, Lawesson's reagent, THF reflux, 18 h, 50%; iii, 4.0 M HCl solution in dioxane, rt, 24 h, 91%; iv, EDC, HOBt, NMM, CH_2Cl_2, 0 °C to rt, 48 h, 68%; v, NaOH, THF, H_2O, rt, 24 h, 88%, vi, 4.0 M HCl solution in dioxane, rt, 18 h, 65% over two steps.

Scheme 11

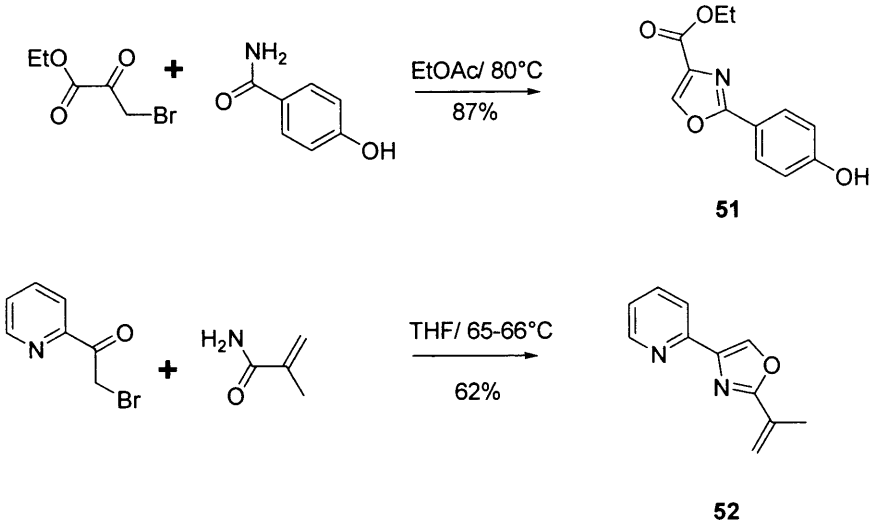


Reagents and conditions: i, HATU, NMM, CH_2Cl_2-DMF (2:1), 0 °C to rt, 72 h, 88%; ii DAST, CH_2Cl_2, -78 °C, 2 h, 88%; iii, MnO_2, CH_2Cl_2, rt, 48 h, 27%;

Scheme 12

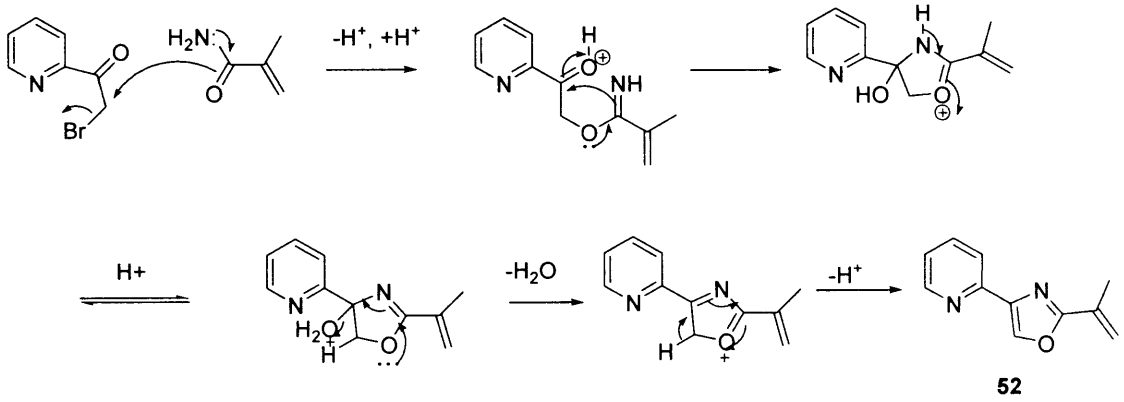
1. 9. 4 2,4-Disubstituted oxazoles by the Hantzsch synthesis

The Hantzsch synthesis of oxazoles involves reaction of an amide with an α-halo ketone, and is one of the most general and reliable routes to 2,4'-disubstituted oxazoles. The commercial availability of the starting materials and its efficiency proves advantageous for parallel synthesis. Reactions using bromoethyl pyruvate appear to require an amide where the carbonyl is attached to a sp^2 hybridised carbon (aromatics, alkenes) as shown in scheme 13. Nelson *et al*[49] successfully prepared an aryl oxazole **51** from an aryl amide and ethyl bromopyruvate on 87% yield. Kelly and Lang[48] also used the Hantzsch approach to prepare **52** as a model for the synthesis of dimethylsulfomycinate. The reaction was carried out by heating methacrylamide with 2-(bromoacetyl) pyridine in THF at reflux, giving **52** in 62% yield (scheme 13).



Scheme 13. Hantzch type routes to bis-oxazoles[48,49]

The mechanism of formation of **52** proceeds via the attack of the amide onto the bromoketone shown in scheme 14.



Scheme 14

Armstrong and co-workers[50] used the Hantzsch synthesis in their approach to Calyculin and related marine natural products (figure 32).

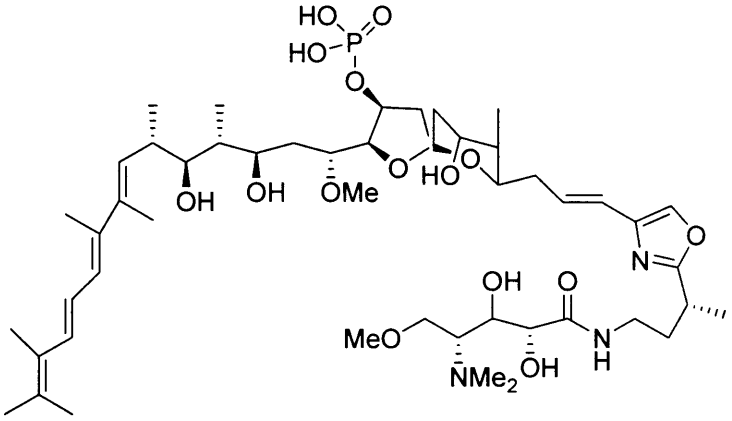
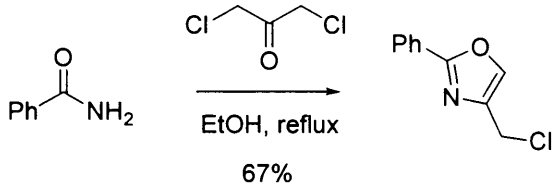


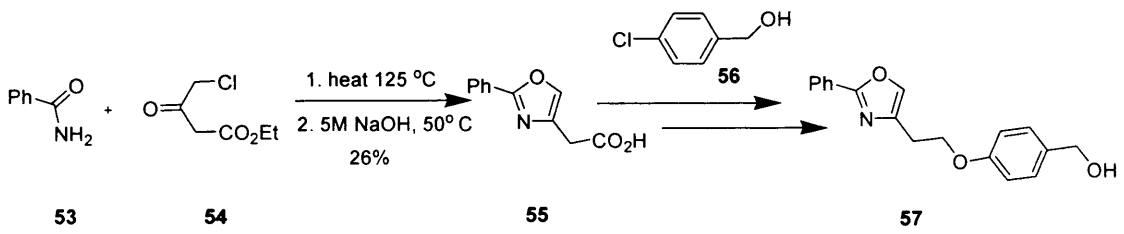
Figure 32 calyculin[51]

They condensed 1,3-dichloroacetone with *sec*-butylamide or benzamide which afforded the 2,4-disubstituted oxazoles (scheme 15). Then they converted the 2,4-disubstituted oxazoles into their desired *trans*-4-alkenyl oxazoles.



Scheme 15

Faul *et al.*[52] used a similar method to Armstrong; they reacted ethyl 4-chloroacetoacetone with benzamide in their preparation of insulin-sensitive enhancers for the treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM). They prepared **55** by reacting benzamide **53** with ethyl 4-chloroacetoacetate **54**. This was then easily converted into the desired oxazole **57** (scheme 16).



Scheme 16

Tris-oxazole units are found in marine natural products that are secondary metabolites, including the ulapualides, halichondramides, mycalolides and kabiramides.

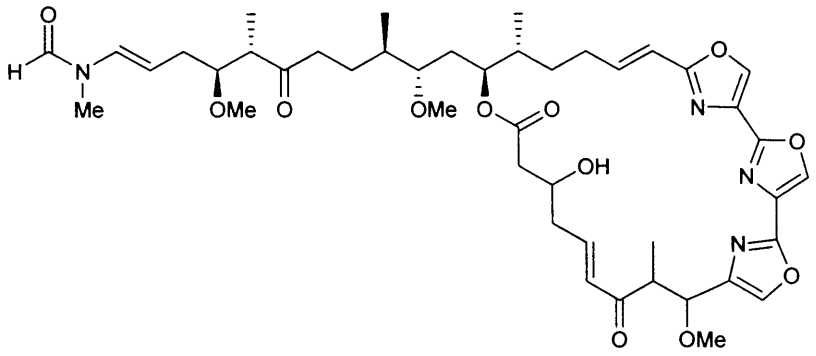
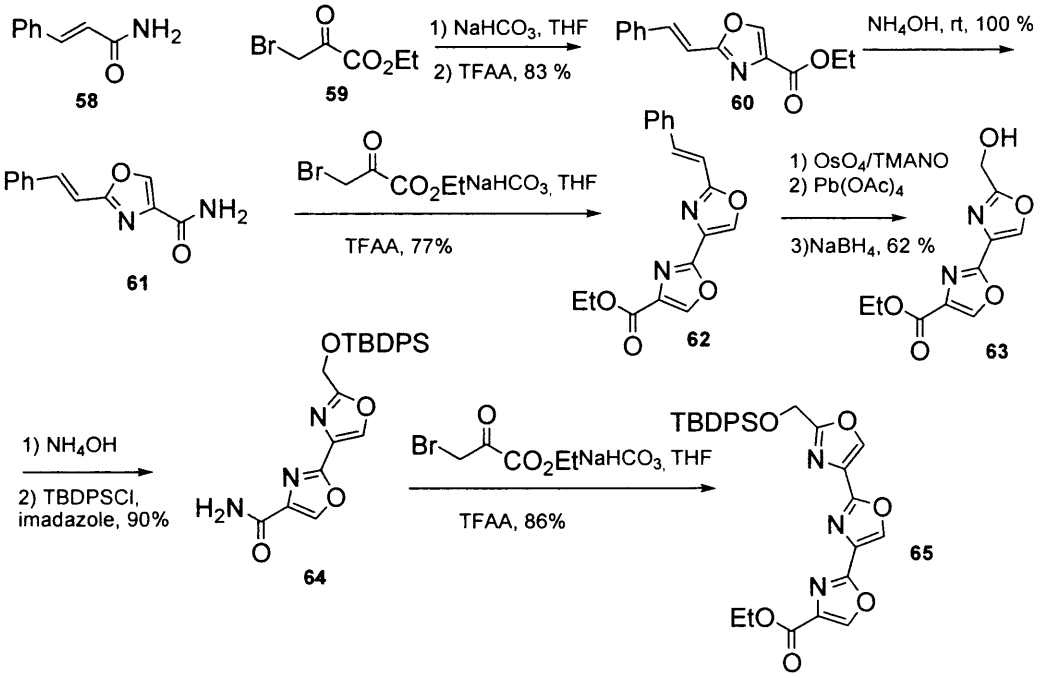


Figure 33. Halichondramide[53]

Panek *et al*[53] attempted the synthesis of the tris-oxazole fragment found in both Halichondramide and Kabiramide C[54]. The synthesis (scheme 17) began with a Hantszch condensation involving cinnamamide and ethyl bromopyruvate. Sodium hydrogen carbonate was also used as a buffer in the reaction to absorb the HBr liberated. TFAA works as the dehydrogenating agents to convert the oxazoline into the oxazole **60**; the Hantzsch method provided an excellent yield of 83%. The terminal ester was then converted quantitatively into the amide using aqueous ammonia. The amide **62** was then condensed with bromo ethylpyruvate to form the bis-oxazole **63** whose ester group was converted into the amide with ammonia, prior to condensation with bromo ethylpyruvate to

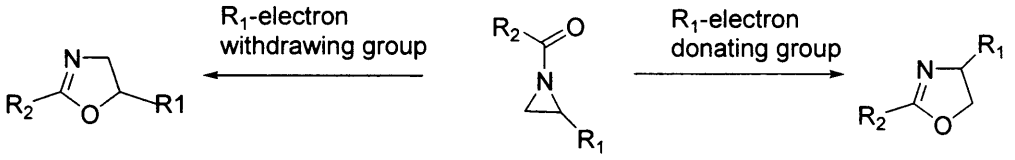
form the tris-oxazole intermediate **65**, common to both natural products. For the 13 steps, an overall yield of 26% was achieved.



Scheme 17

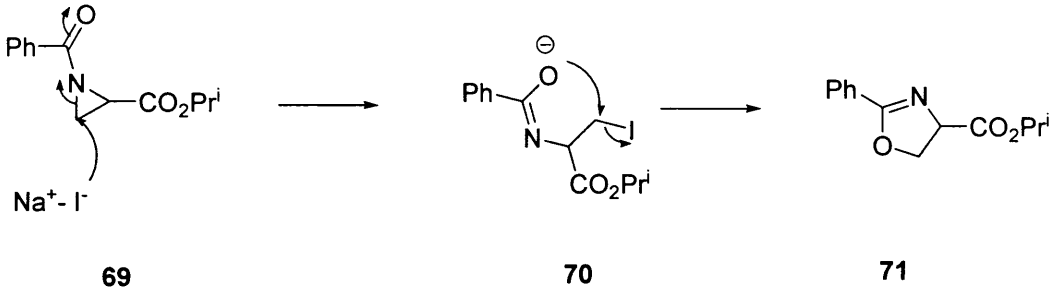
1. 9. 5 2,4-Disubstituted oxazoles from *N*-acylazridines

Eastwood *et al*[55,56] was also interested in the synthesis of Halichondramide. This group investigated the rearrangement of *N*-acylaziridines carrying substituents which could allow for possible bis- and tris-oxazole fragments. Eastwood *et al.* developed a regioselective method to synthesise 2,4-disubstituted oxazoles from *N*-acylaziridines. 2-Positioned *N*-acylazridines were chosen to undergo a ring expansion to form the oxazoline. Eastwood found that the R group on the aziridine must be electron-donating in order to form a 2,4-disubstituted oxazole. If the R group is electron-withdrawing, a 2, 5-disubstituted oxazole is obtained (scheme 18).



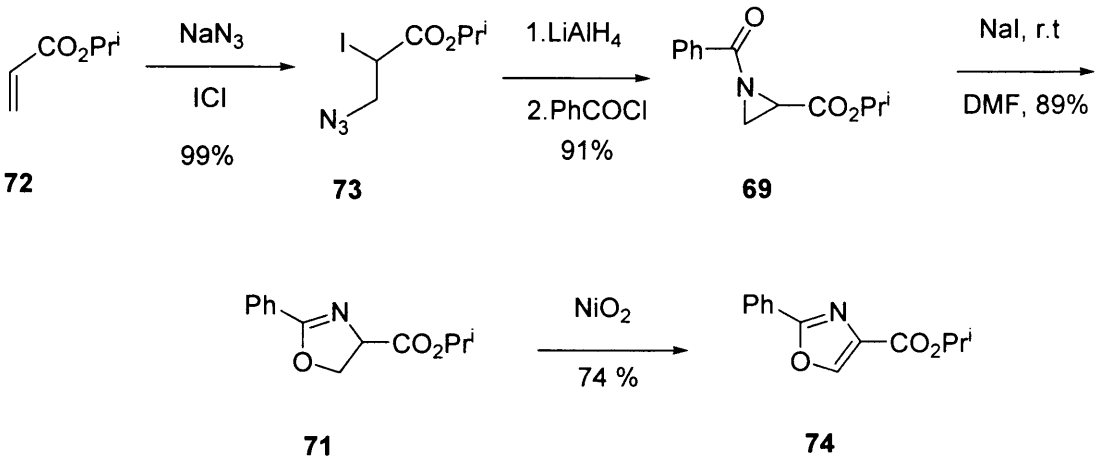
Scheme 18

The proposed mechanism of formation of oxazoline **71** begins by the iodo anion species attacking the less hindered carbon atom of the aziridine **69**, leading to the imidate ion **70**. This oxy-anion then attacks the CH_2 position displacing iodide (scheme 19).



Scheme 19

The ring-expansion of the aziridine was conducted in DMF at room temperature (scheme 20) using NaI. A good yield (89%) was obtained; however, some 2,5-substituted product was also formed. Dehydrogenation using NiO_2 then afforded the oxazoline providing a 74%.



Scheme 20

The Williams-Wipf protocol seems to be the most general route by far, although the Hantzsch is the least costly and most favoured on a large scale.

References

(1) Neidle, S. *Chem. in Britain* **2000**, *36*, 27-29.

(2) Wattenberg, L. W. *Cancer Res.* **1985**, *45*, 1-8.

(3)http://stemcells.nih.gov/StaticResources/info/scireport/images/figurea1.jpg.

(4) Hopson, L. J.; Wessells, N. K. *Essential Biology,*158.

(5) Schwartz, G. K.; Shah, M. A. *J. Clin. Oncol.* **2005**, *23*, 9408-9421.

(6) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 799.

(7) L. Michaelis, M. M. *Biochem. Z.* **1913**, *49*, 333-369.

(8) Lineweaver, H. a. B. *J. Am. Chem. Soc.* **1934**, *56*, 658-666.

(9) Gomez, D.; O'Donohue, M. F.; Wenner, T.; Douarre, C.; Macadre, J.; Koebel, P.; Giraud-Panis, M. J.; Kaplan, H.; Kolkes, A.; Shin-Ya, K.; Riou, J. F. *Cancer Res.* **2006**, *66*, 6908-6912.

(10) Blasco, M. A. *Cancer Lett.* **2003**, *194*, 183-188.

(11) Hiyama, E.; Hiyama, K. *Cancer. Lett.* **2003**, *194*, 221-233.

(12) Lee, H. W.; Blasco, M. A.; Gottlieb, G. J.; Horner, J. W.; Greider, C. W.; DePinho, R. A. *Nature* **1998**, *392*, 569-574.

(13) Counter, C. M.; Hahn, W. C.; Wei, W. Y.; Caddle, S. D.; Beijersbergen, R. L.; Lansdorp, P. M.; Sedivy, J. M.; Weinberg, R. A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14723-14728.

(14) Zglinicki, T. v. *Cancer Lett.* **2003**, *194*, 137-138.

(15) Sasaki, S.; Ehara, T.; Sakata, I.; Fujino, Y.; Harada, N.; Kimura, J.; Nakamura, H.; Maeda, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 583-585.

(16) Nakamura, T. M.; Morin, G. B.; Chapman, K. B.; Weinrich, S. L.; Andrews, W. H.; Lingner, J.; Harley, C. B.; Cech, T. R. *Science* **1997**, *277*, 955-959.

(17) Saretzki, G. *Cancer Lett.* **2003**, *194*, 209-219.

(18) Kondo, L.; Kondo, Y.; Li., G. *Oncogene* **1998**, *16*, 3323-3330.

(19) Zhang, X. M. V. *Genes Dev.* **1999**, *13*, 2388-2399.

(20) McClintock, B. *Genetics* **1941**, *26*, 234-282.

(21) McClintock, B. *Proc. Natl. Acad. Sci USA* **1939**, *24*, 404-416.

(22) Blackburn, E. H.; Gall, J. G. *J. Mol. Biol.* **1978**, *120*, 33-53.

(23) Greider, C. W.; Blackburn, E. H. *Cell* **1985**, *43*, 405-413.

(24) Harrington, L. *Cancer. Lett.* **2003**, *194*, 139-154.

(25) Blackburn, L. H. *Cell* **1982**, *29*, 245-255.

(26) Yin, F.; Liu, J. H.; Peng, X. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3923-3926.

(27) Zahler, A. M.; Williamson, J. R.; Cech, T. R.; Prescott, D. M. *Nature* **1991**, *350*, 718-720.

(28) Shi, D. F.; Wheelhouse, R. T.; Sun, D. Y.; Hurley, L. H. *J. Med. Chem.* **2001**, *44*, 4509-4523.

(29) Sun, D. Y.; Thompson, B.; Cathers, B. E.; Salazar, M.; Kerwin, S. M.; Trent, J. O.; Jenkins, T. C.; Neidle, S.; Hurley, L. H. *J. Med. Chem.* **1997**, *40*, 2113-2116.

(30) Kim, N. W.; Harley, C. B.; Prowse, K. R.; Weinrich, S. L.; Piatyszek, M. A.; Wright, W. E.; Shay, J. W. *Science* **1995**, *268*, 1116-1117.

(31) Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L. C.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. *Science* **1994**, *266*, 2011-2015.

(32) Maraval, A.; Franco, S.; Vialas, C.; Pratviel, G.; Blasco, M. A.; Meunier, B. *Org. Biomol. Chem.* **2003**, *1*, 921-927.

(33) Dai, F.; Perez, J.; Reszka, A. P.; Chessari, G.; Read, M. A.; Burger, A. M.; Neidle, S. *Eur. J. Cancer* **2002**, *38*, S90-S91.

(34) Neidle, S.; Read, M. A. *Biopolymers* **2000**, *56*, 195-208.

(35) Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177-1180.

(36) Schmitz, F. J.; Deguzman, F. S.; Hossain, M. B.; Vanderhelm, D. *J. Org. Chem.* **1991**, *56*, 804-808.

(37) Warabi, K.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *J. Org. Chem.* **2003**, *68*, 2765-2770.

(38) Shin-ya, K.; Wierzba, K.; Matsuo, K.; Ohtani, T.; Yamada, Y.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Am. Chem. Soc.* **2001**, *123*, 1262-1263.

(39) Kim, M. Y.; Vankayalapati, H.; Kazuo, S.; Wierzba, K.; Hurley, L. H. *J. Am. Chem. Soc.* **2002**, *124*, 2098-2099.

(40) Minhas, G. S.; Pilch, D. S.; Kerrigan, J. E.; LaVoie, E. J.; Rice, J. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3891-3895.

(41) Doyle, K. J.; Moody, C. J. *Tetrahedron* **1994**, *50*, 3761-3772.

(42) Connell, R. D.; Tebbe, M.; Gangloff, A. R.; Helquist, P.; Akermark, B. *Tetrahedron* **1993**, *49*, 5445-5459.

(43) Wang, Y.; Janjic, J.; Kozmin, S. A. *J. Am. Chem. Soc.* **2002**, *124*, 13670-13671.

(44) Cardwell, K. S.; Hermitage, S. A.; Sjolin, A. *Tetrahedron Lett.* **2000**, *41*, 4239-4242.

(45) Deeley, J.; Pattenden, G. *Chem.Commun.* **2005**, 797-799.

(46) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.

(47) Williams, D. R.; Brooks, D. A.; Berliner, M. A. *J. Am. Chem. Soc.* **1999**, *121*, 4924-4925.

(48) Kelly, T. R.; Lang, F. R. *J. Org. Chem.* **1996**, *61*, 4623-4633.

(49) Nelson, K. A. *U. S. Pat.* **1978**, *5849922*.

(50) Zhao, Z. C.; Scarlato, G. R.; Armstrong, R. W. *Tetrahedron Lett.* **1991**, *32*, 1609-1612.

(51) Pihko, P. M.; Koskinen, A. M. P. *J. Org. Chem.* **1998**, *63*, 92-98.

(52) Faul, M. M.; Winneroski, L. L.; York, J. S.; Reinhard, M. R.; Hoying, R. C.; Gritton, W. H.; Dominianni, S. J. *Heterocycles* **2001**, *55*, 689-704.

(53) Liu, P.; Celatka, C. A.; Panek, J. S. *Tetrahedron Lett.* **1997**, *38*, 5445-5448.

(54) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M.; Noguchi, H.; Sankawa, U. *J. Org. Chem.* **1989**, *54*, 1360-1363.

(55) Eastwood, F. W.; Perlmutter, P.; Yang, Q. *J. Chem. Soc. Perk Trans. 1* **1997**, 35-42.

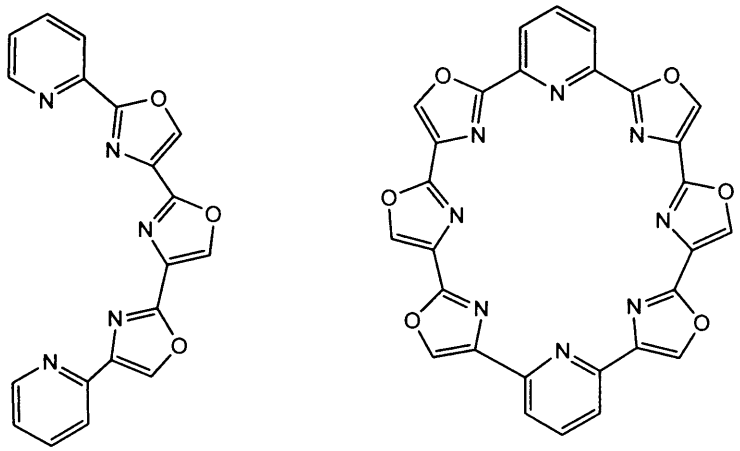
(56) Eastwood, F. W.; Perlmutter, P.; Qi, Y. *Tetrahedron Lett.* **1994**, *35*, 2039-2042.

Chapter 2

2.0 Approaches to the synthesis of Polyoxazoles 75 and 76

2.1 Introduction

One of the aims of this project was to synthesise a capped polyoxazole **75** and a similar macrocyclic polyoxazole system such as **76** (fig 34) which can eventually be evaluated for specific binding to telomeric DNA G–quadruplexes and inhibitors of the human telomerase enzyme. The structure of **76** resembles telomestatin being planar, electron deficient and containing multiple oxazole rings, and especially an internal planar location of eight sp^2-hybridised nitrogen atoms.



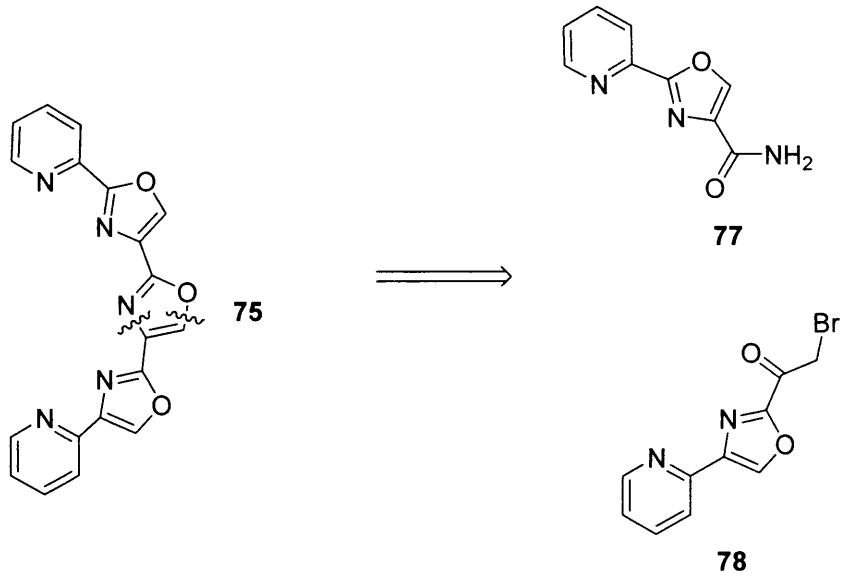
75 **76**

Figure 34. Polyoxazole targets

2. 2 Synthetic Approaches to Dipyridyltrisoxazole 75

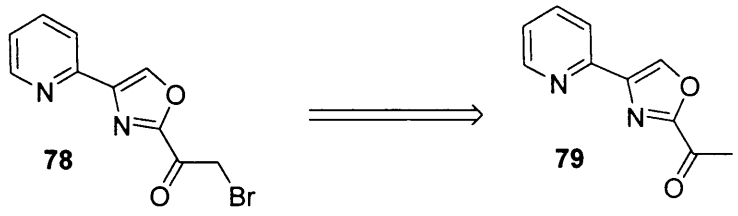
2.2.1 Retrosynthetic Analysis of Dipyridyltrisoxazole 75

From a retrosynthetic view point the synthesis of **75** would require the following intermediates shown in scheme 21. It was decided that the central oxazole rings of **75** should be disconnected to form two fragments which include the amide **77** and the bromoketone **78** for the subsequent Hantzsch reaction discussed in chapter one.



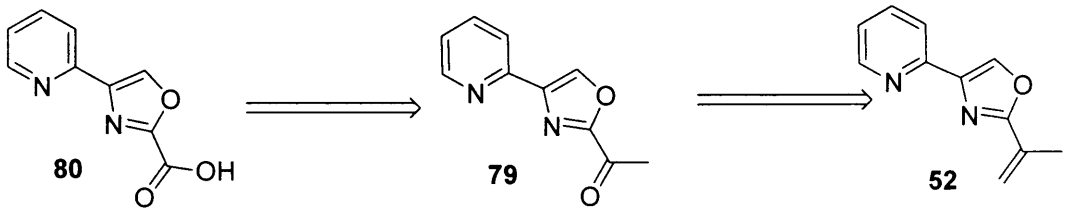
Scheme 21

The bromoketone **78** can be formed by a bromination reaction using molecular bromine and acetic acid on the ketone **79** (scheme 22).



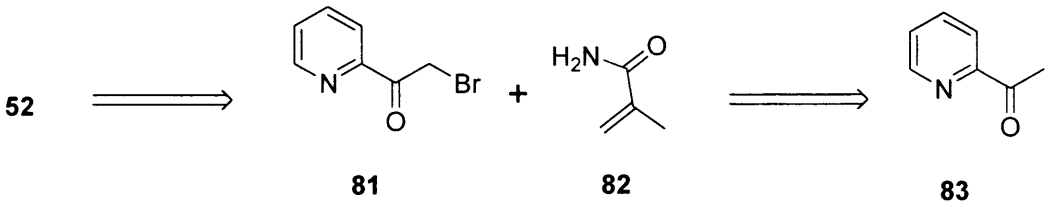
Scheme 22

A haloform reaction[1,2] on the ketone **79** using NaOBr would provide the carboxylic acid **80**. The ketone **79** could be synthesised by ozonolysis of the alkene **52**.[3]



Scheme 23

Another Hantzsch type reaction involving 2-bromoacetylpyridine (**81**) and meth-acrylamide **82** in a sealed tube can synthesise olefinic oxazole **52** (scheme 23).[3] The bromoketone **81** can be synthesised from 2-acetylpyridine **83** (scheme 24).[4,5]

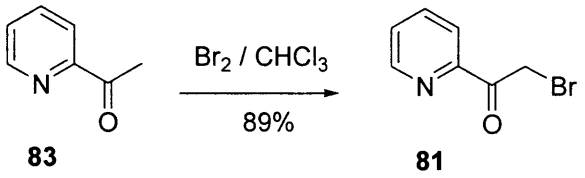


Scheme 24

2. 2. 2 Approaches to the synthesis of Dipyridyltrisoxazole 75

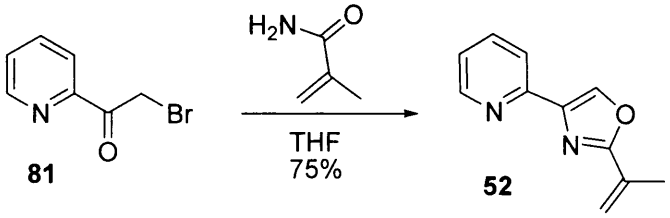
2. 2. 3 An approach via the Hantzsch condensation

The synthesis of **75** required the synthesis of the intermediate bromo ketone **81**. 2-Acetyl pyridine **83** was reacted with bromine in chloroform under reflux to give pyridyl bromoketone **81** with a high yield (scheme 25). [6]



Scheme 25

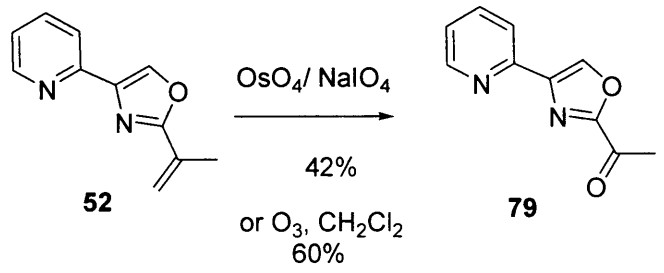
The attempted synthesis of the olefinic pyridyl oxazole **52** began with a Hantzsch reaction. The reagents used were pyridyl bromoketone **81**, very soluble in dichloromethane, and methacrylamide, heated in a sealed tube (scheme 26). After 2 days at 100 °C, the mixture was evaporated to leave a brown oil. The tlc of the reaction (Rf 0.5 EtOAc) showed a luminous blue spot which was isolated in 75% yield using column chromatography and NMR had shown it to be the oxazole **52**.[3]



Scheme 26

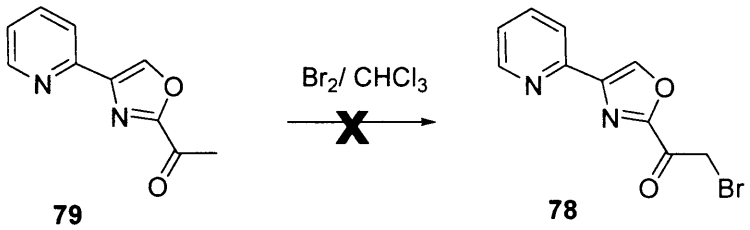
Oxidation of **52** using osmium tetraoxide and sodium periodate gave ketone **79** (scheme 27). The yield of this reaction was very low, mainly because of the work-up. It was found that the ketone **79** partitioned between the organic

and aqueous layers; presumably the sodium ion chelates to the nitrogen atom of the pyridine ring and the nitrogen atom of the oxazole. An alternative reaction involving ozonolysis of the alkene **52** to give the ketone **79** was carried out to avoid the problem of chelation, and gave an improved yield of 60%. [3]



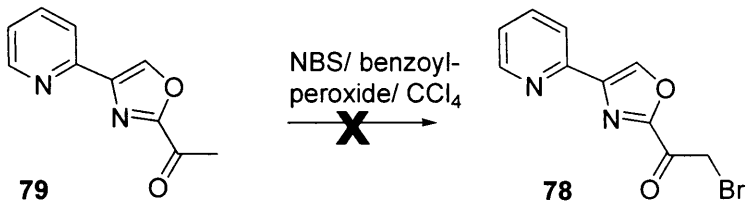
Scheme 27

Preparation of 2-bromo-1-(4-pyridin-2-yl-oxazol-2-yl)-ethanone (**78**) proved quite difficult. Procedures carried out involved reacting bromine with the corresponding ketone **79** at reflux using chloroform for 24 hours (scheme 28).[6] There was no reaction taking place; therefore, alternative conditions using a 100 W light bulb together with heat were tried, but to no avail. Chloroform was then replaced by dioxane in order to increase the temperature of the reaction; however, the increase in energy did not cause any products to form. Acetic acid was also used as the solvent, but with no success.[4]



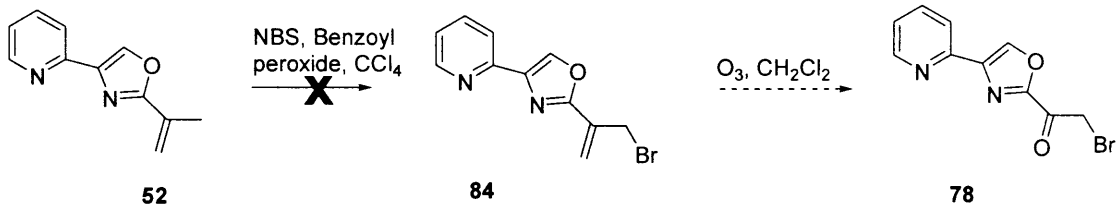
Scheme 28

In order to prepare the bromoketone **78** a radical reaction was attempted on the ketone **79** in the presence of benzoyl peroxide and recrystallised *N*-bromosuccinimide in carbon tetrachloride (scheme 29).[7] The mixture was heated under reflux for more than 48 hours; however, tlc showed no reaction had taken place. A 100 W lamp was then placed near the reaction flask, also in the presence of benzoyl peroxide to initiate the radical process, but again no reaction took place. Benzoyl peroxide was then replaced by the initiating reagent TMSOTf, [8] but nor was that successful.



Scheme 29

Another radical-based route was then attempted, involving bromination of the alkene **52**. Once obtained, the allylic bromide **84** would be converted into the ketone via ozonolysis to give **78** (scheme 30).[9] Tlc of the reaction mixture showed a new spot; the ^{1}H NMR spectrum showed that the alkene peaks had disappeared but that the methyl group remained intact; hence, some attack of bromine on the alkene had taken place.

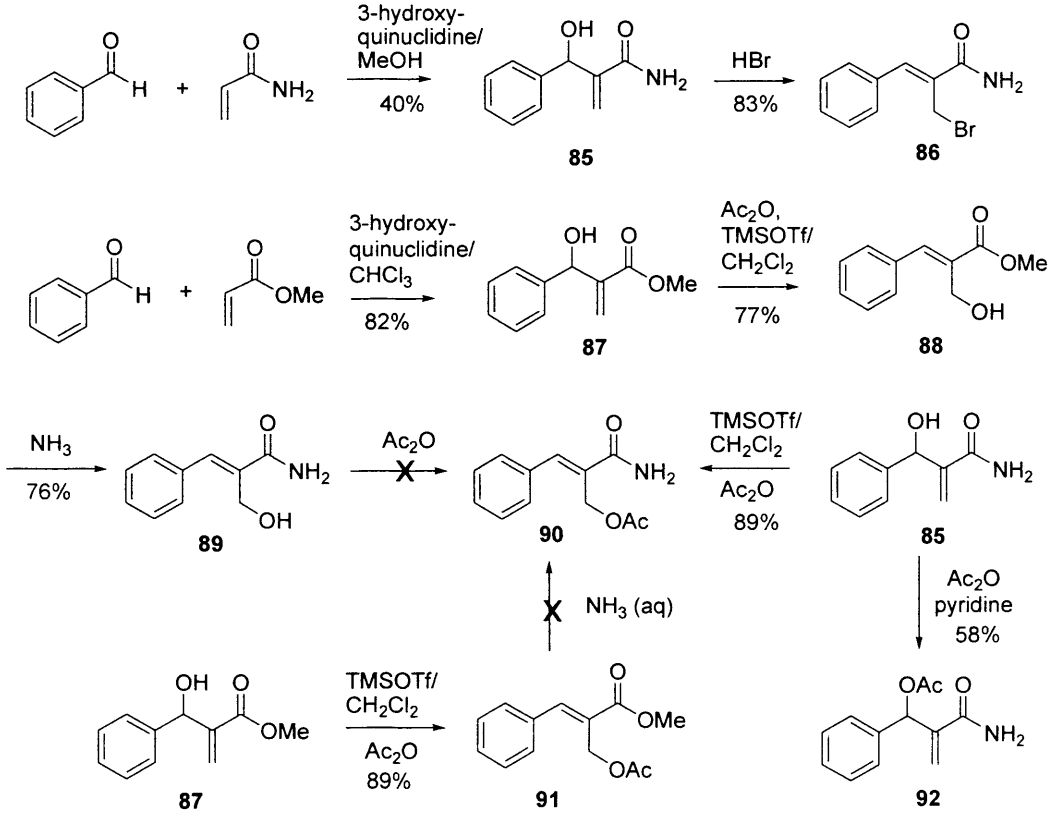


Scheme 30

In summary, none of the above attempts was successful. It appears that bromination of a methyl ketone attached to an oxazole ring is difficult, and not much is mentioned in the literature, despite the fact that the corresponding acyl pyridines can be readily brominated.[10,11,12]

Owing to the inability to obtain **78** directly from the ketone **79** an alternative route was devised, involving intermediates prepared from Baylis-Hillman reactions (scheme 31), which was efficient, high-yielding and required inexpensive reagents. The products also underwent allylic isomerize to give **86**,

91[13] and **88**[14] again in good yields. The next step was to perform a Hantzsch reaction using the corresponding isomerised amides **90**, **85**,[15] **89**,[16] **92**, with the pyridyl bromoketone **81**.

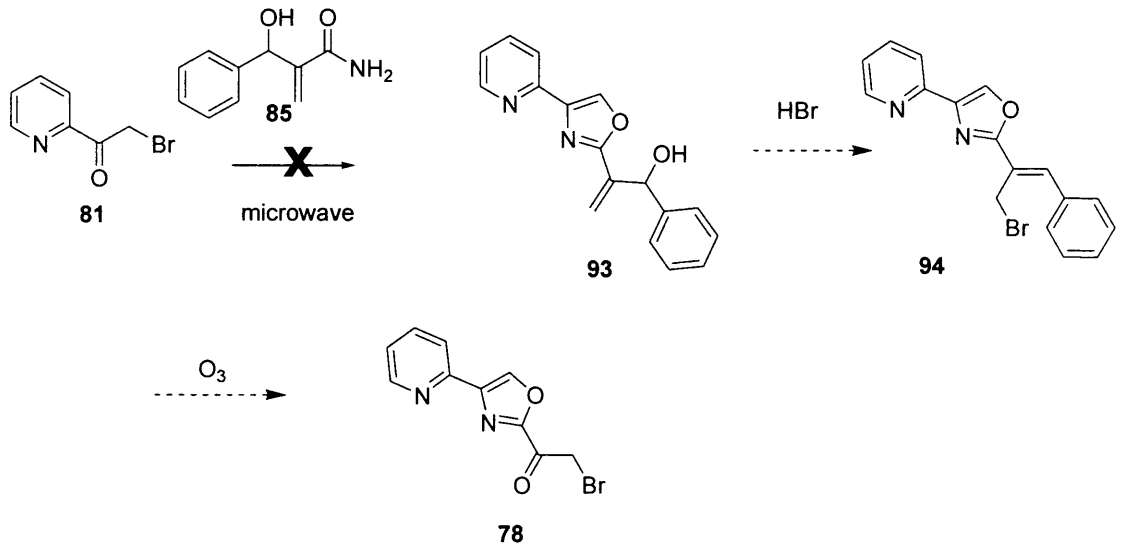


Scheme 31

Compounds **85**, **86** and **89** (schemes 32, 33 and 34) were used in the Hantzsch reaction in order to try to obtain the key bromo ketone intermediate **78**.

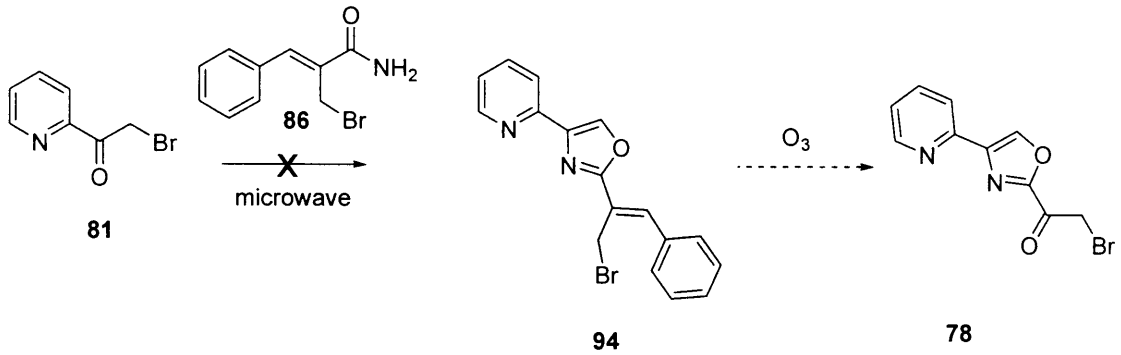
Firstly, the amide **85** was reacted with the bromoketone **81** in a sealed tube for 2 days (scheme 32). It was thought that if the oxazole **93** was obtained then a bromination reaction would take place to form the allylic bromide **94**; a subsequent reagent would cleave the phenyl group. However, the tlc of the reaction showed 6 spots which each were isolated but none proved to be the Hantzsch product. The conditions were modified; for example THF was replaced

by dioxane and microwave irradiation maintained at 150 W, but the reaction did not succeed.



Scheme 32

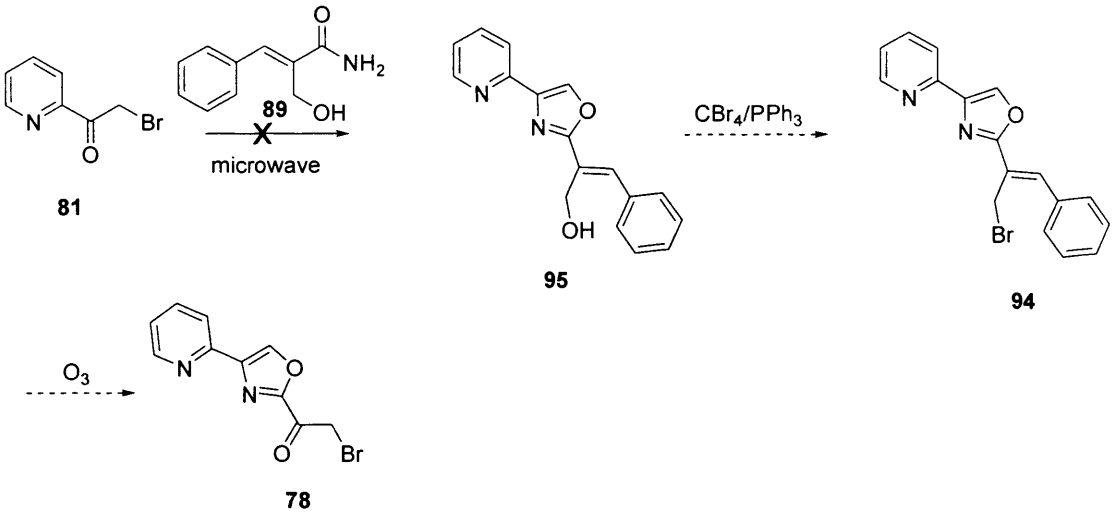
Another attempt to obtain bromoketone **78** was made using amide **86**. The amide **86** and bromoketone **81** in THF were irradiated with microwaves for 5 minutes at 150 W (scheme 33). However, tlc showed no reaction had taken place as there was only starting material found. Ozonolysis of the alkene **94** was expected to furnish bromoketone **78**.



Scheme 33

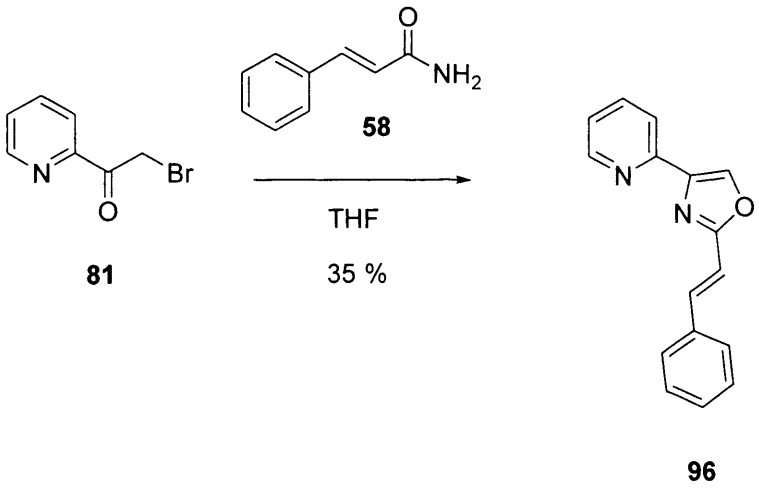
A Hantzsch reaction was attempted on the amido alcohol **89** and bromoketone **81** via irradiation with microwaves (scheme 34). The Hantzsch reaction was unsuccessful as the tlc showed many spots, none proving to be the

product **95**. If successful, the alcohol **95** would have been converted into the bromide **94** using carbon tetrabromide and triphenylphosphine followed by ozonolysis to give the key intermediate **81**.



Scheme 34

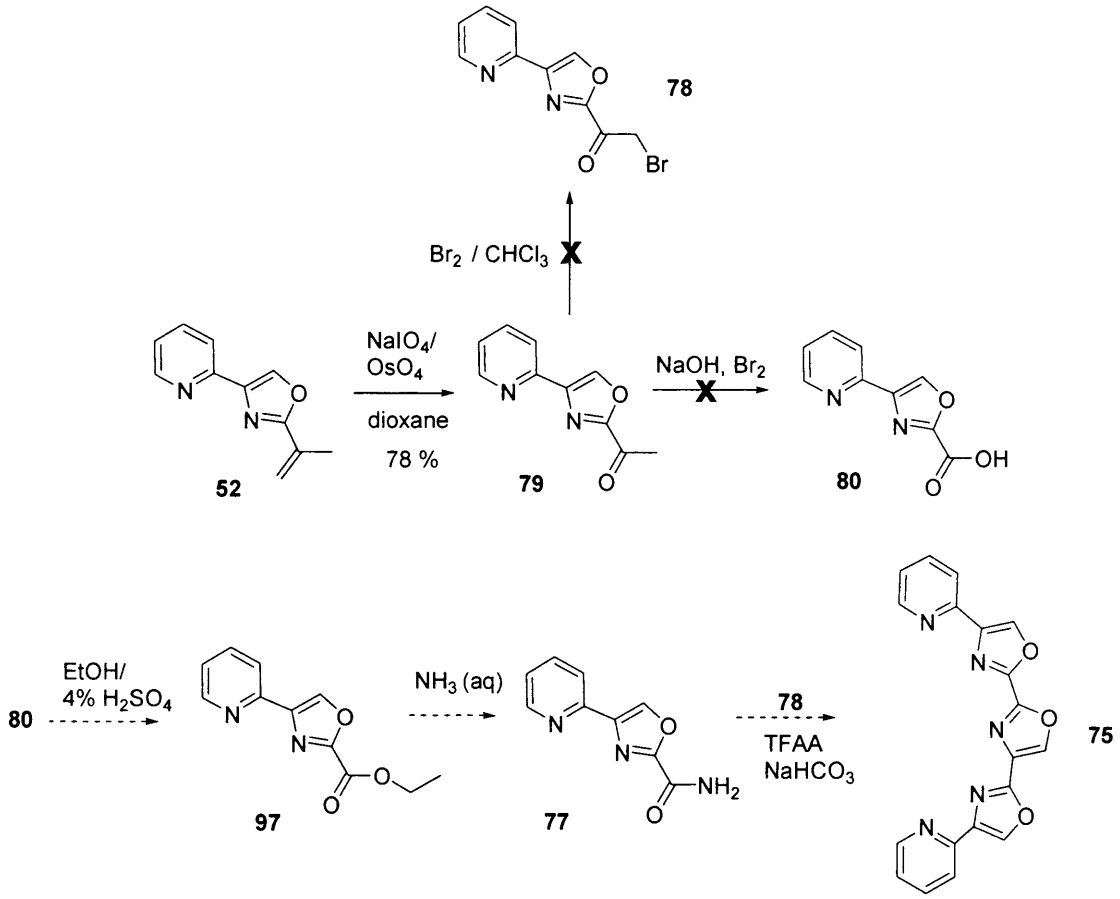
The only compound made via a Hantzsch synthesis was oxazole **96**, prepared from cinnamamide (**58**) and the pyridyl bromoketone **81** (scheme 35). The product **96** was formed by placing the two reactants dissolved in THF into a sample tube, then irradiating it in a microwave for 5 minutes. Tlc visualised under UV showed a new bright blue luminous spot which was isolated by column chromatography using neat ethyl acetate.



Scheme 35

An attempt was made to cleave off the phenyl group by ozonoylsis, to oxazole **96** was dissolved in dichloromethane and the temperature was lowered prior to the passage of ozone. After 30 minutes the reaction mixture was quenched with DMS; tlc showed no reaction had taken place since only the starting material **96** was present.

The carboxy-oxazole **80** (scheme 36) was needed in order to prepare the amide **77** required for the Hantzsch reaction. The synthesis of the mono-oxazole acid **80** from the ketone **79** was attempted using sodium hydroxide and bromine. Tlc of the reaction mixture showed baseline product, characteristic of an acid. However, the weight of the crude material once evaporated to dryness was too high and it would only dissolve in water, not in DMSO or chloroform, suggesting contamination of the desired product with inorganic salts. The inorganic salts were difficult to separate from the product, so an alternative route to synthesise **77** was investigated.

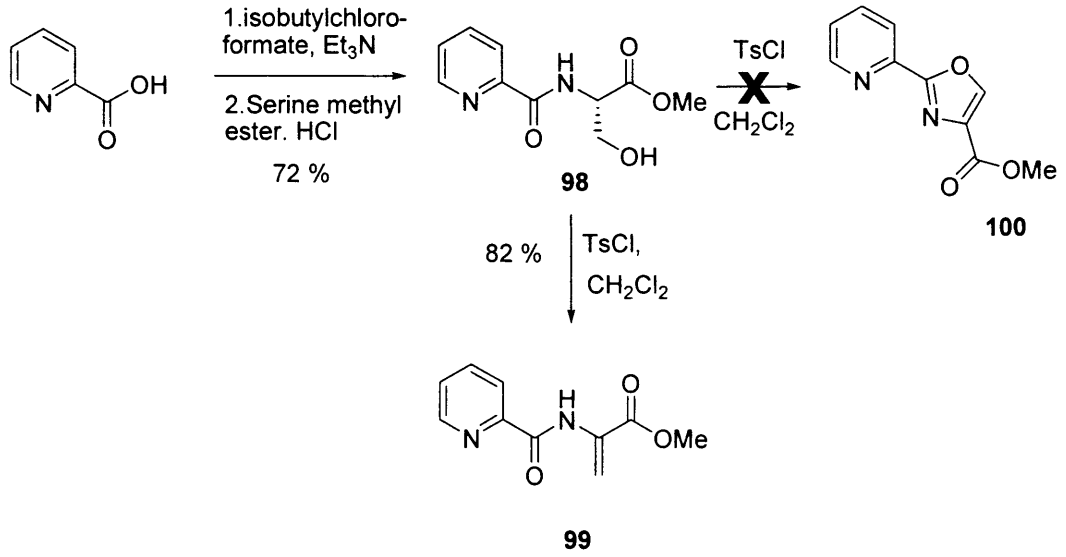


Scheme 36

2. 2. 4 An approach via the Williams-Wipf condensation

The route to **75** began with the coupling of picolinic acid with serine methyl ester hydrochloride to form the amide **98**[17] in 75% yield (scheme 37). The reagents used were 1.1 equivalents of isobutyl chloroformate coupling agent in the presence of triethylamine.

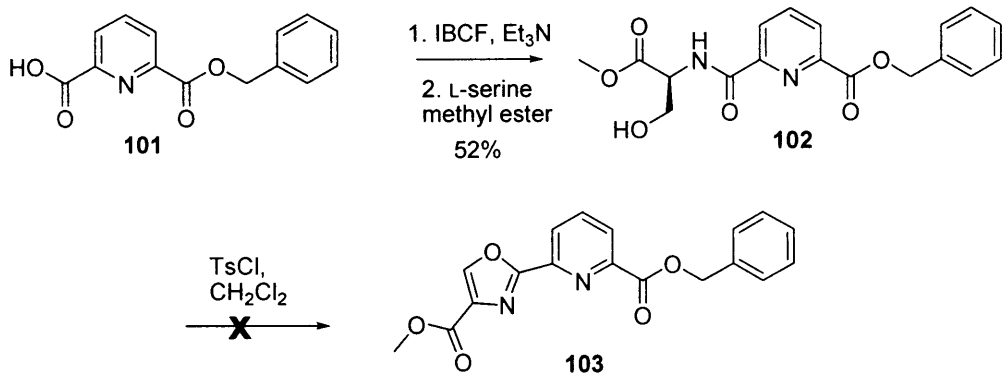
The reaction was carried out at -30 °C and then stirred at ambient temperature for 17 hours. Tlc showed two spots, both of which were isolated; one at Rf 0.7 (ethyl acetate) which was found to be the dehydro amide **99**, and the lower spot at Rf 0.3, shown to be the desired amido alcohol **98,** which was of a high yield with no major problems.



Scheme 37

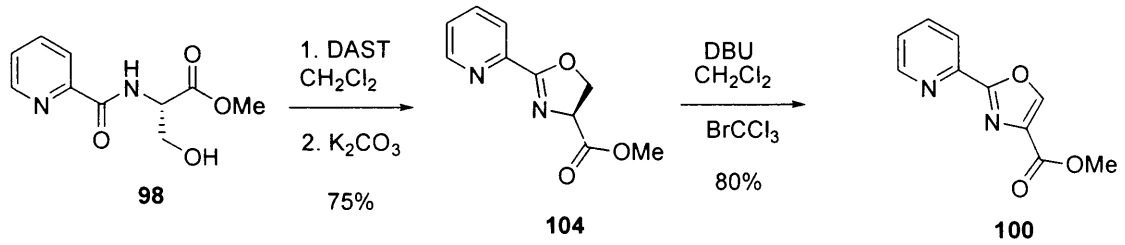
The next step required the cyclisation of amido alcohol **98** to give the oxazole **100**. The procedure involved stirring the amide with TsCl and triethylamine at room temperature for two days. ^{1}H NMR data showed that an elimination reaction took place to give the dehydroamide, signals being observed for a new double bond at δ 5.65. Cyclisation may have failed because of the deactivating (carboxyl) group leading instead to the eliminated product **99**[18] (scheme 37).

Other L-serine derived compounds (scheme 38) were used to investigate oxazole ring formation, but to no avail.



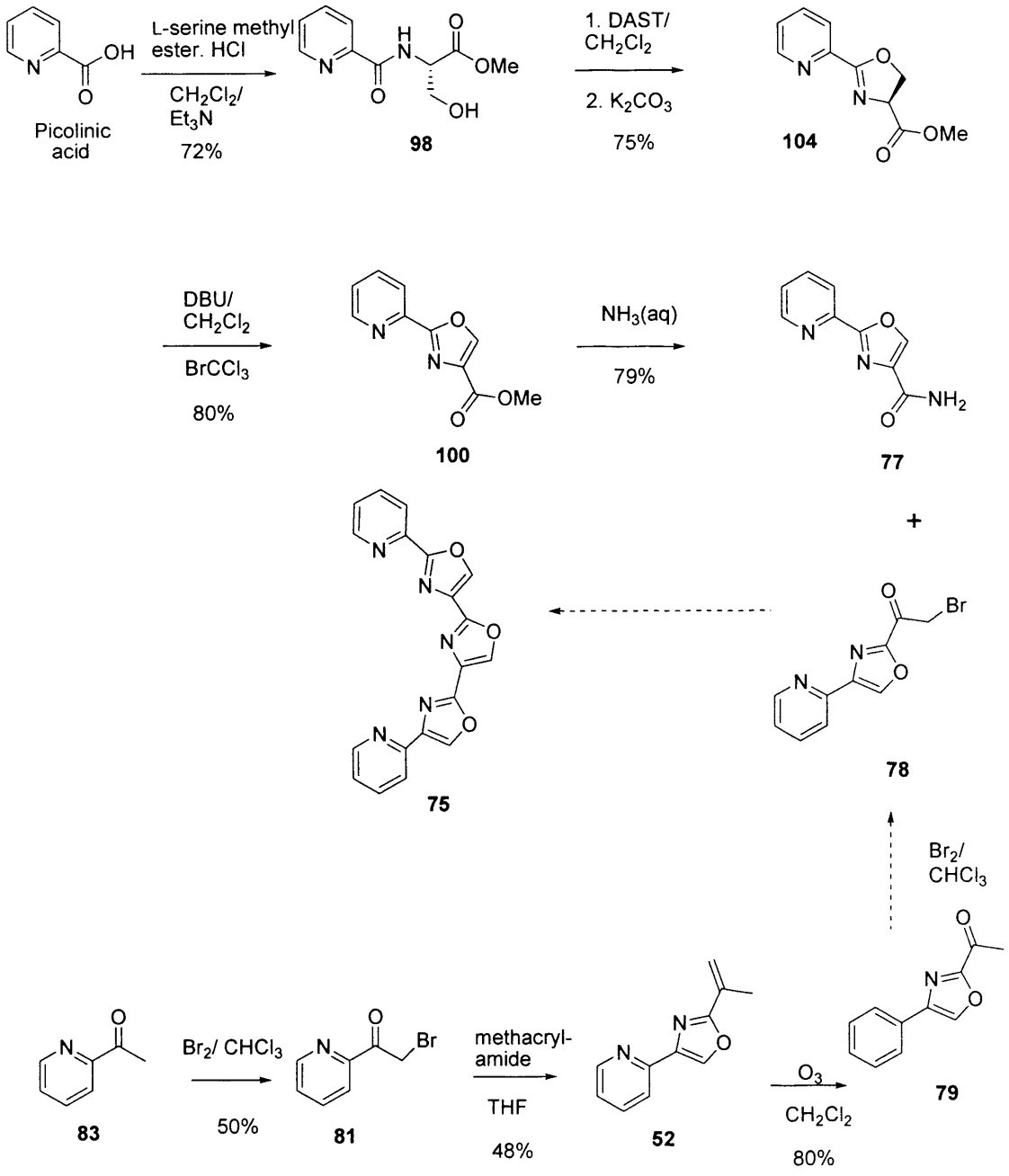
Scheme 38

The amido alcohol **98** was then successfully converted into the oxazoline **104** using a Williams-Wipf procedure[19] which required DAST at -78 °C (described in chapter 1). The oxazoline **104** was then converted into the oxazole **100** using DBU and bromotrichloromethane, with no significant side-products (scheme 39).



Scheme 39

The second fragment required for the final Hantzsch reaction to obtain pentacycle **75** was the amide **77** which was prepared by treatment of the methyl ester **100** with aqueous ammonia (35% yield). Amide **77** precipitated almost immediately and was filtered and washed with water to give material sufficiently clean for an attempted Hantzsch reaction.

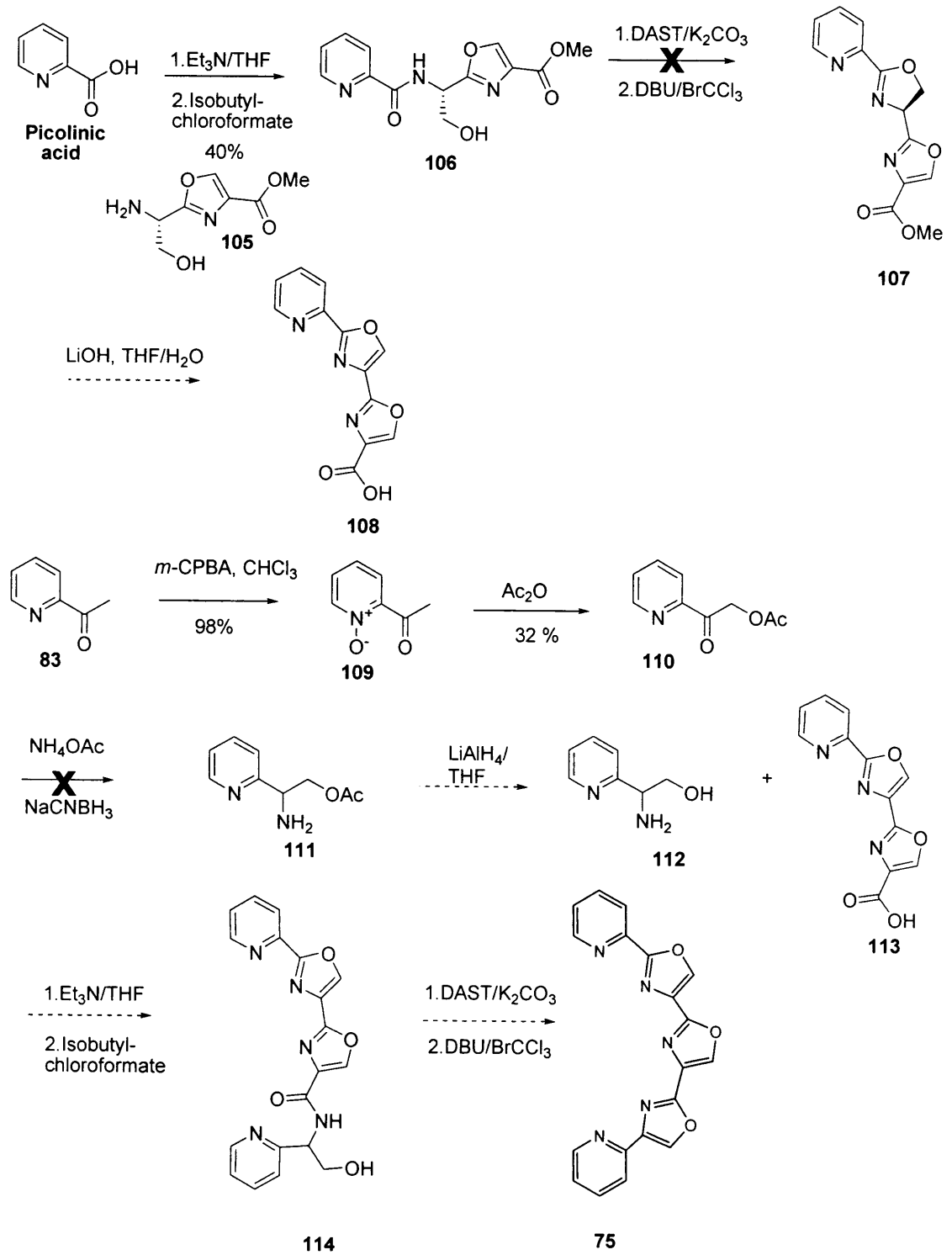


Scheme 40

In summary, the key amide intermediate **77** was synthesised but the bromoketone partner **78** could not be prepared, and so since the Hantszch reaction could not be attempted, an alternative route was investigated.

A revised route to **75** was proposed (scheme 41). Coupling of 2-picolinic acid with the oxazole **105** using isobutyl chloroformate and triethylamine (scheme 41) afforded amide **106**; the ^{1}H NMR spectrum confirmed product and the HRMS data for the parent ion were within 1 ppm of that expected. The next step involved the cyclisation to the oxazoline using DAST and potassium carbonate to give **107**; however, many side-products formed and with little or no oxazoline detected. The results may have indicated that the reaction had been left for too long, so a shorter time stirring for 2 instead of 16 hours at room temperature, but tlc still showed many spots.

The synthesis to **75** required the alcohol **112.** The synthesis of **112** began by reacting 2-acetylpyridine with *m*-CPBA in chloroform to give the pyridyl *N*-oxide[20] **109** in high yield (98%) in scheme 41. The pyridyl *N*-oxide **109** was then converted to 2-oxo-2-(pyridine-2-yl)ethylacetate **110** using acetic anhydride. The product was very difficult to purify owing to the gelatinous property of component **110** which made column chromatography very difficult and hence furnishing a low yield (32%). Many attempts were made to convert the keto group **110** into the amine **111** using ammonium acetate and sodium cyanoborohydride but that proved unsuccessful as the tlc only showed starting material. The route to **75** was abandoned due to many problems in the synthesis.

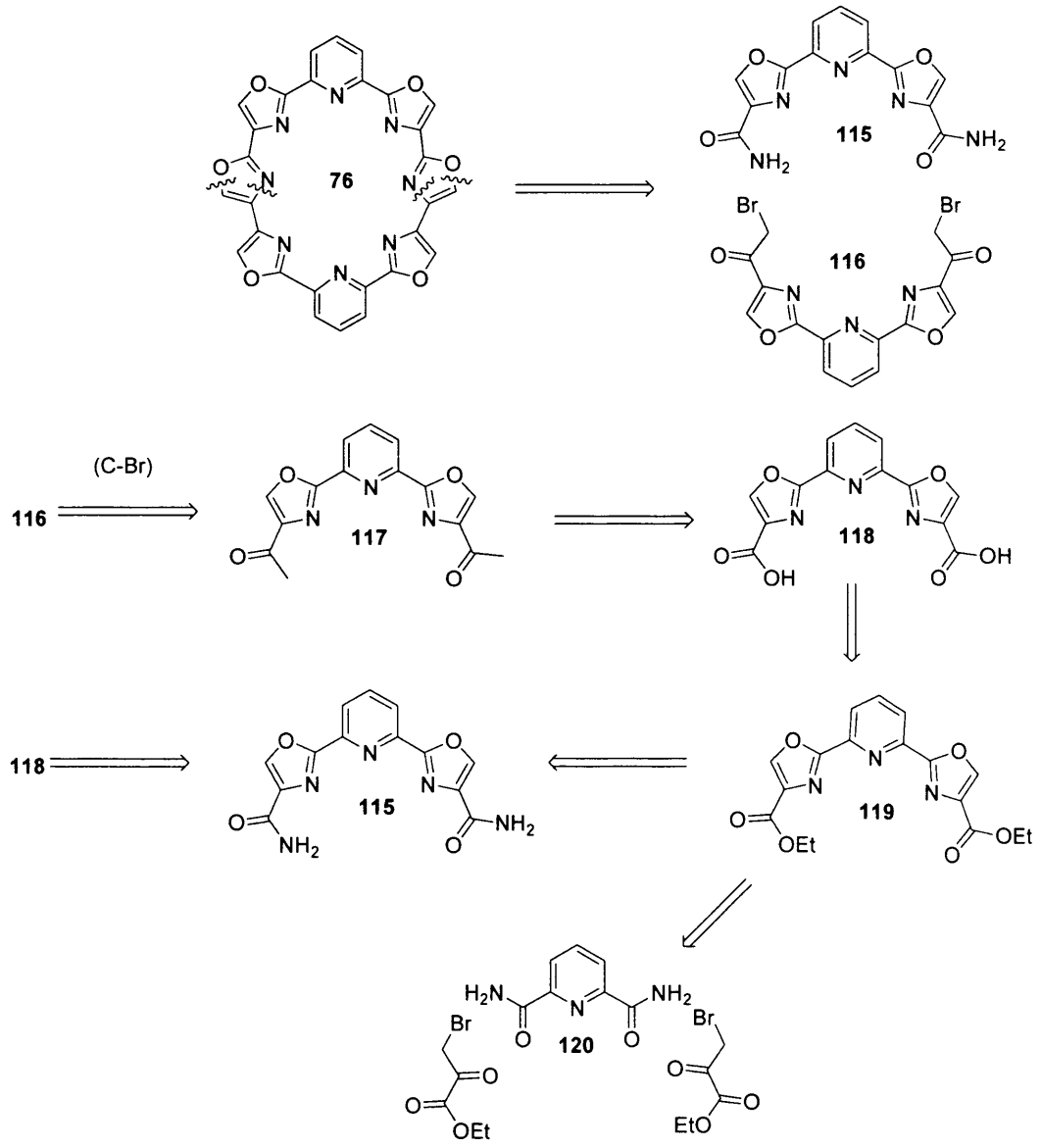

Picolinic acid
1.Et3N/THF
2.Isobutyl-chloroformate
40%
106
105
1.DAST/K2CO3
2.DBU/BrCCl3
107
LiOH, THF/H2O
108
m-CPBA, CHCl3
98%
83
109
Ac2O
32 %
110
NH4OAc
NaCNBH3
111
LiAlH4/ THF
112
113
1.Et3N/THF
2.Isobutyl-chloroformate
114
1.DAST/K2CO3
2.DBU/BrCCl3
75

Scheme 41

2. 3 Synthetic approach to the Dipyridyl macrocycle 76

2. 3. 1 Retrosynthetic analysis of Dipyridyl macrocycle 76

From a retrosynthetic view point the synthesis of **76** would require the intermediates shown in scheme 42. It was decided that the central oxazole rings in **76** should be disconnected to form two fragments which include the bisamide **115** and the bromo ketone **116** for condensation in a Hantzsch reaction.



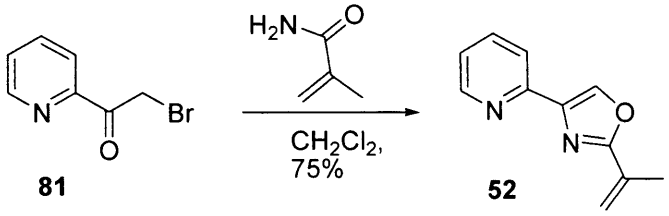
Scheme 42

The bromoketone **116** can be formed by a bromination reaction using molecular bromine in acetic acid on the diketone **117**, which can be prepared from the diacid **118** by treatment with methyllithium in anhydrous THF at -78 °C (scheme 42). Hydrolysis of the diester **119** was expected to give the diacid **118**. Another Hantzsch type reaction involving pyridine-2,6-dicarboxamide **120** and ethyl bromopyruvate was hoped to give diester **119.** The second major fragment required the diamide **115** which can be formed by amination of the ester **119**.

2. 3. 2 Approaches to the synthesis of Dipyridyl macrocycle 76

2. 3. 3 An approach via the Hantzsch condensation

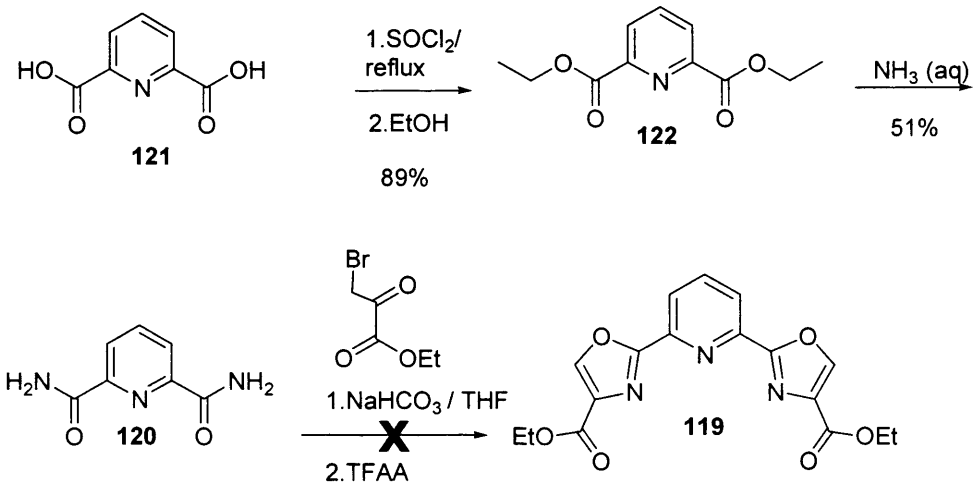
Intially, the modified Hantzsch reaction was pursued in an attempt to obtain the bisoxazolylpyridine **119**, proceeding via pyridine 2,6-dicarboxamide **120** (scheme 44).[21] However, many problems were found with the Hantzsch reaction. First, pyridine 2,6-dicarboxamide (**120**) was very insoluble in most solvents except DMF, the solubility preventing the reaction. Accordingly, the oxazolyl pyridine **52** (scheme 43) was selected as the target, in order to minimise problems with solubility and to provide a better understanding of how to construct each oxazole ring.



81 **52**

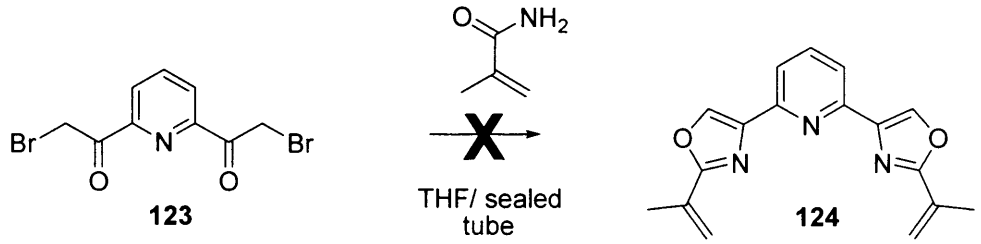
Scheme 43

To date, using the Hantzsch reaction to prepare an oxazole has been quite difficult, apparently because ethyl bromopyruvate decomposes upon heating; optimal conditions for the reaction have not been identified, despite many attempts.



Scheme 44

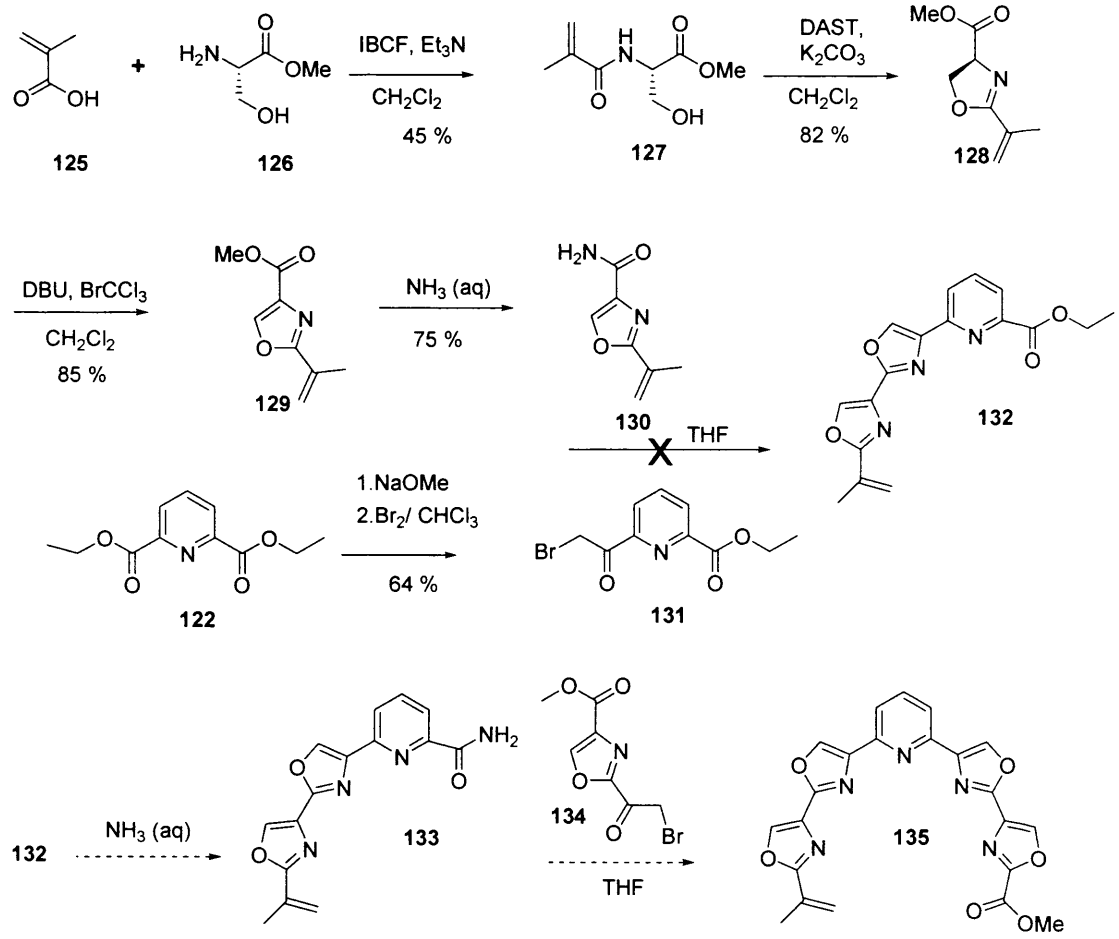
It was hoped that the protocol for the synthesis of monooxazole **52** could be applied to the bisoxazole. Similar conditions were chosen for the reaction carried out with the monooxazole **52** whereby the bromopyridyl ketone **81**[12] and methacrylamide were heated in THF in a sealed tube for 5 days at 75 °C (scheme 45). The tlc results looked promising as a new spot comprised of Rf 0.5 (EtOAc) was found and the starting material had been consumed. An attempt was made to isolate the new spot using column chromatography; however, the material decomposed. The reaction proved to be unsatisfactory because many spots were formed, probably indicating polymerisation.



Scheme 45

Another attempt was made to synthesise the bispyridyl macrocycle **76**. Since double-Hantzsch had not worked; a mono-Hantzsch approach was considered mindful of previous success (scheme 46).

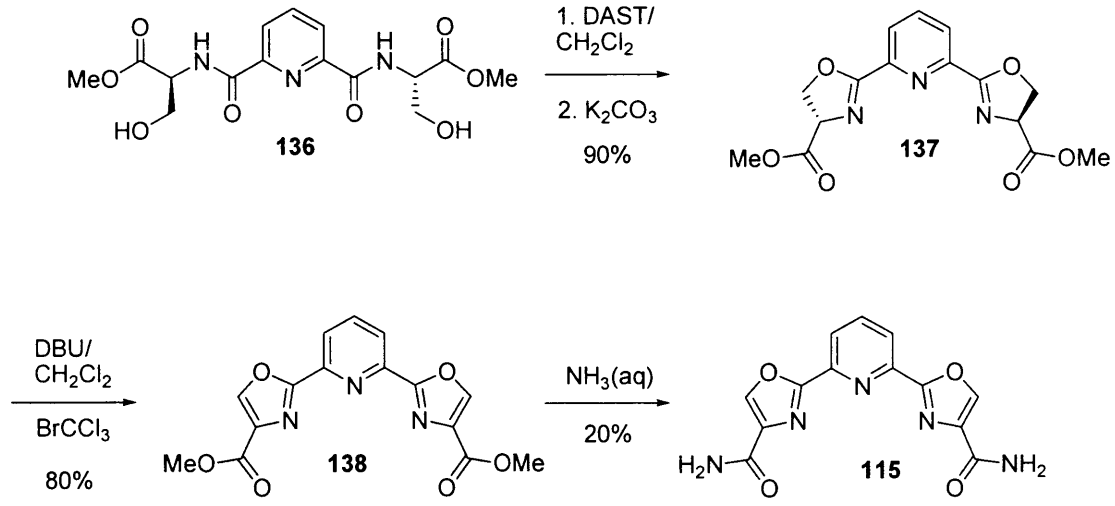
The new route required the synthesis of amido-oxazole **130** for reaction with the bromo ketone **131** (scheme 46). The amide **127** was synthesised from L-serine methyl ester hydrochloride **126** and methacrylic acid **125**. The amide **127** was then converted into the oxazoline **128** using the Williams and Wipf procedure[22] providing a high yield of 82%. The oxazoline **128** was then dehydrogenated to the oxazole **129** with DBU and bromotrichloromethane in a 85% yield. The ester group was converted into the amide **130** using aqueous ammonia (0.880), in a yield of 75%. The starting bromoketone **131** contained an ester group, which could be converted into an amide in order, to attempt a further Hantzsch reaction with the corresponding bromoketone **134** to form the polyoxazole macrocycle. The amide **130** and the bromoketone **131** were dissolved in THF and stirred for 16 hours under reflux. However, tlc showed no reaction had taken place.



Scheme 46

2. 3. 4 An approach via the Willams-Wipf condensation

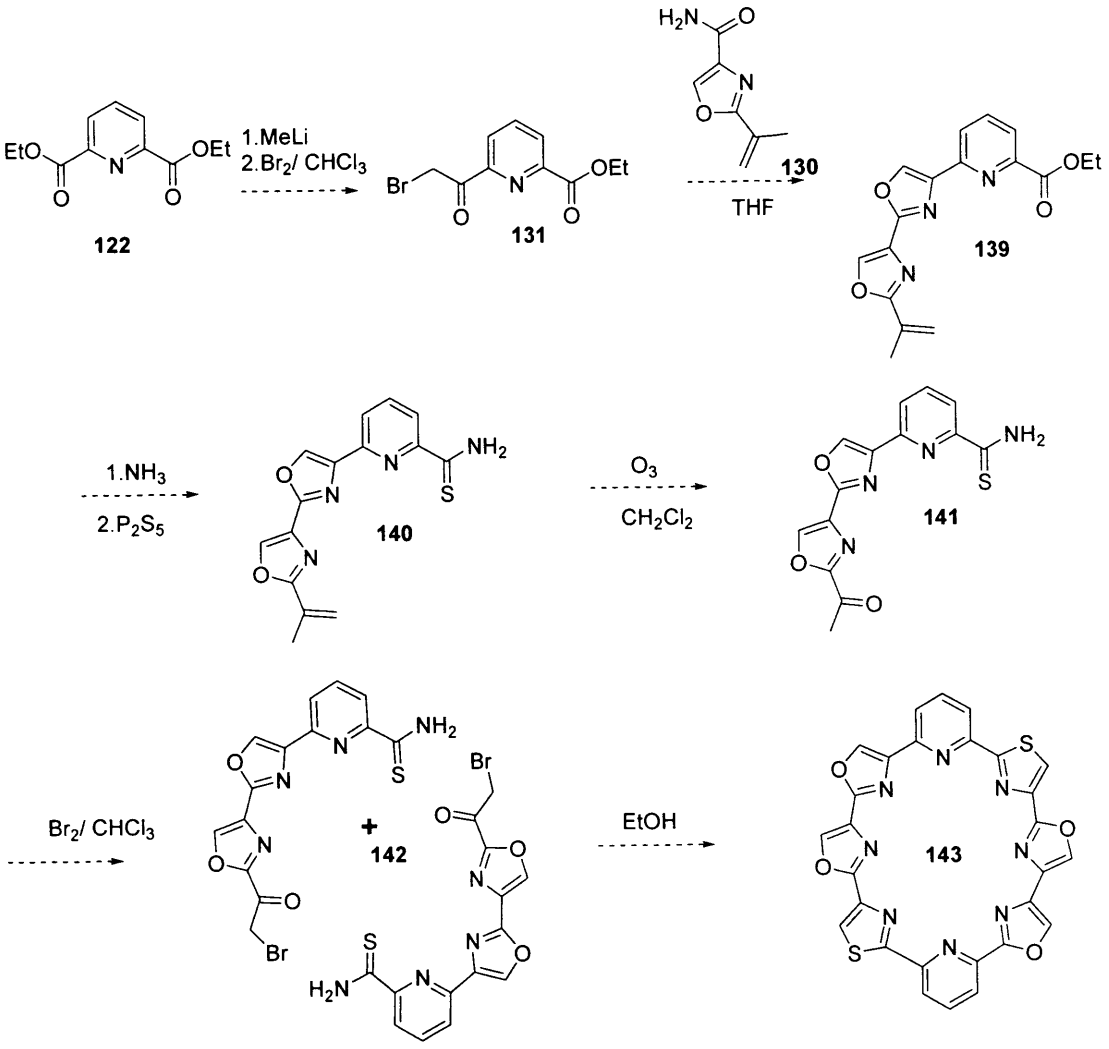
In view of the problems encountered with the Hantzsch approach to oxazoles an alternative route to the diamide **115** was sought. A literature procedure using the diester **136**[23] was found by which it was cyclised to form the bisoxazoline **137**. The cyclisation occured using DAST in the presence of potassium carbonate and sodium hydrogen carbonate, a procedure pioneered by Williams and Wipf[22] (scheme 47). The pyridyl diester **136** was prepared in 64% yield from the diacid chloride of pyridine-2,6-dicarboxylic acid and L-serine methyl ester using 4.0 equivalents of triethylamine. The ^{1}H NMR spectrum showed a new peak at δ 4.84, corresponding to the methine signals in **136**. The diamide **136** was converted into the oxazoline **137** by using 2.2 equivalents of DAST. ^{1}H NMR data showed that the reaction was successful, since the N-H peak was not present and the CH_2 peak had shifted to a multiplet at 4.69 ppm. The sequence was then taken further than the literature to give the bisoxazole **138**, in a net dehydrogenation reaction using 4.0 equiv of DBU and 2.1 equiv of bromotrichloromethane. This reaction was successful, the CH_2 peaks being absent and a new oxazolyl singlet peak at δ 8.40 ppm (2H) appearing. The ester **138** was then converted into the amide **115** by stirring at room temperature with 0.880 aqueous ammonia (scheme 47) but in only 20% yield due to incompleteness of the reaction. The NMR spectrum of the bisamide **115** showed the absence of a methyl ester signal but the presence of a broad singlet at 7.83 ppm. The diamide is one of the two fragments required for the synthesis of macrocycle **76** (scheme 42).



Scheme 47

In summary, only one of the two fragments, the diamide **115**, was synthesised as required for a Hantzsch reaction to give macrocycle **76.** Formation of a bromo ketone from an oxazolyl methyl ketone seemed very difficult, and the synthesis had to be abandoned.

Future work could include a new route to an eight-membered macrocycle (scheme 48). However, rather than having six oxazole rings the macrocycle could comprise of four oxazoles and two thiazole rings. Thiazoles are more readily formed than are oxazoles during the Hantzsch reaction.


EtO
OEt
1.MeLi
2.Br2/ CHCl3
122
Br
131
H2N
130
THF
139
1.NH3
2.P2S5
140
NH2
S
O3
CH2Cl2
141
Br2/ CHCl3
142
EtOH
143

Scheme 48

References

(1) Lama, M.; Mamula, O.; Scopelliti, R. *Synlett* **2004**, 1808-1810.

(2) Ciufolini, M. A.; Valognes, D.; Xi, N. *Angew. Chem. Int. Ed.* **2000**, *39*, 2493.

(3) Kelly, T. R.; Lang, F. R. *J. Org. Chem.* **1996**, *61*, 4623-4633.

(4) Barlin, G. B.; Davies, L. P.; Ireland, S. J.; Ngu, M. M. L. *Austr. J. Chem.* **1989**, *42*, 1735-1748.

(5) Ruano, J. L. G.; Pedregal, C.; Rodriguez, J. H. *Tetrahedron* **1987**, *43*, 4407-4416.

(6) Imuta, M.; Kawai, K.; Ziffer, H. *J.Org. Chem.* **1980**, *45*, 3352-3355.

(7) Huo, M.; Kuang, Y. Y.; Chen, F. E. *Org. Prep. Proced. Int.* **2004**, *36*, 331-335.

(8) Guha, S. K.; Wu, B.; Kim, B. S.; Baik, W.; Koo, S. *Tetrahedron Lett.* **2006**, *47*, 291-293.

(9) Flekhter, O. B.; Giniyatullina, G. V.; Galin, F. Z.; Baschenko, N. Z.; Makara, N. S.; Zarudii, F. S.; Boreko, E. I.; Savinova, O. V.; Pavlova, N. I.; Starikova, Z. A.; Tolstikov, G. A. *Chem. Nat. Compds.* **2005**, *41*, 706-709.

(10) Pinori, M. L., Maria; Modena, Daniela; Mascagni, Paolo. *PCT Int. Appl. 2005040156* **2005**.

(11) Callahan, J. F.; Burgess, J. L.; Fornwald, J. A.; Gaster, L. M.; Harling, J. D.; Harrington, F. P.; Heer, J.; Kwon, C.; Lehr, R.; Mathur, A.; Olson, B. A.; Weinstock, J.; Laping, N. J. *J. Med. Chem.* **2002**, *45*, 999-1001.

(12) Deady, L. W.; Stanborough, M. S. *Aust. J. Chem.* **1981**, *34*, 1295-1302.

(13) Basavaiah, D.; Muthukumaran, K.; Sreenivasulu, B. *Synthesis* **2000**, 545-548.

(14) Basavaiah, D.; Padmaja, K.; Satyanarayana, T. *Synthesis* **2000**, 1662-1664.

(15) Faltin, C.; Fleming, E. M.; Connon, S. J. *J. Org. Chem.* **2004**, *69*, 6496-6499.

(16) Leadbeater, N. E.; van der Pol, C. *J. Chem. Soc., Perkin. Trans. 1* **2001**, 2831-2835.

(17) Ayida, B. K.; Simonsen, K. B.; Vourloumis, D.; Hermann, T. *Bioorg. Med. Chem.Lett.* **2005**, *15*, 2457-2460.

(18) Shin, C. G.; Okumura, K.; Ito, A.; Nakamura, Y. *Chem. Lett.* **1994**, 1305-1306.

(19) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.

(20) Nagano, H.; Hamana, M.; Nawata, Y.; Prachayasittikul, S.; Abdelsayed, A. N.; Bauer, L. *Heterocycles* **1987**, *26*, 1263-1270.

(21) McElvain, S. *J. Am. Chem. Soc* **1935**, *57*, 1135.

(22) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.

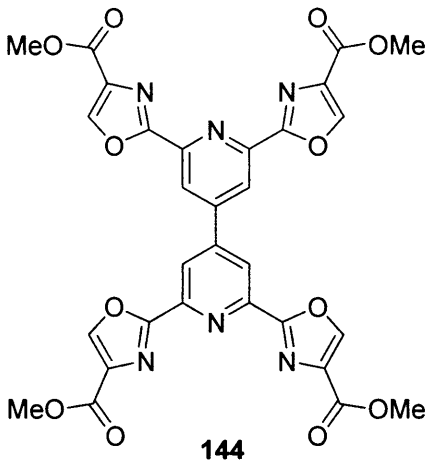
(23) Iwasa, S.; Nakamura, H.; Nishiyama, H. *Heterocycles* **2000**, *52*, 939-944.

Chapter 3

3.0 Approaches to Oxazolylpyridines

3.1 Introduction

One of the aims of this project was to synthesise a planar polyoxazole system such as **144** (fig 35) which could be evaluated for specific binding to telomeric DNA G–quadruplexes and as an inhibitor of the enzyme human telomerase. The structure consists of a bipyridyl ring system to which four oxazole rings are attached. The assembly contains nitrogen atoms in the centre of the ring system that could form intermolecular hydrogen bonds with G-quadruplexes.

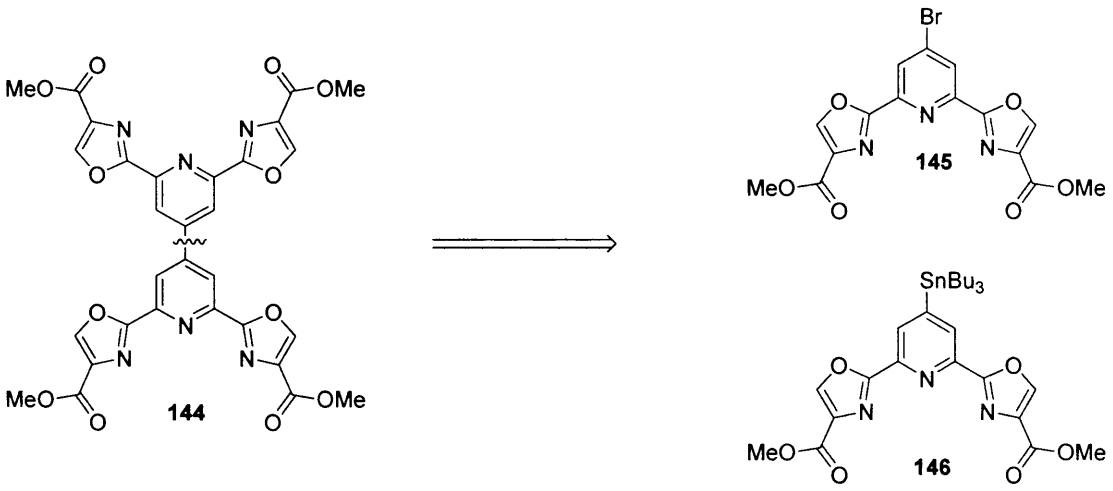


144

Figure 35.

3. 2 Retrosynthetic analysis of the tetraoxazolyl bipyridyl 144

It was decided that the bipyridyl ring system should be disconnected first to form two fragments which include the bromopyridine **145** and the pyridylstannane **146**, to be joined via a Stille coupling.



Scheme 49

In order to obtain stannane **146**, via a Stille coupling, a transmetalation reaction[1] is required which involves an organotin species reacting with a halide in the presence of palladium as the catalyst. The reaction mechanism has been well studied (figure 36).[2, 3] The initial step in the catalytic cycle involves the reduction of a palladium (II) species **147** to the active palladium (0) species, **148**. The next step, oxidative addition, involves addition of the halide species **149** to the palladium (0) species, oxidising it to form palladium (II) intermediate **150**.[4]

Transmetalation occurs from the stannane **160** to the palladium (II) species **161**, giving the palladium species **162**. Finally, a reductive elimination process takes place, in which the desired product **163** is eliminated and the catalytic cycle is perpetuated palladium (0) through the release of the species **148**.

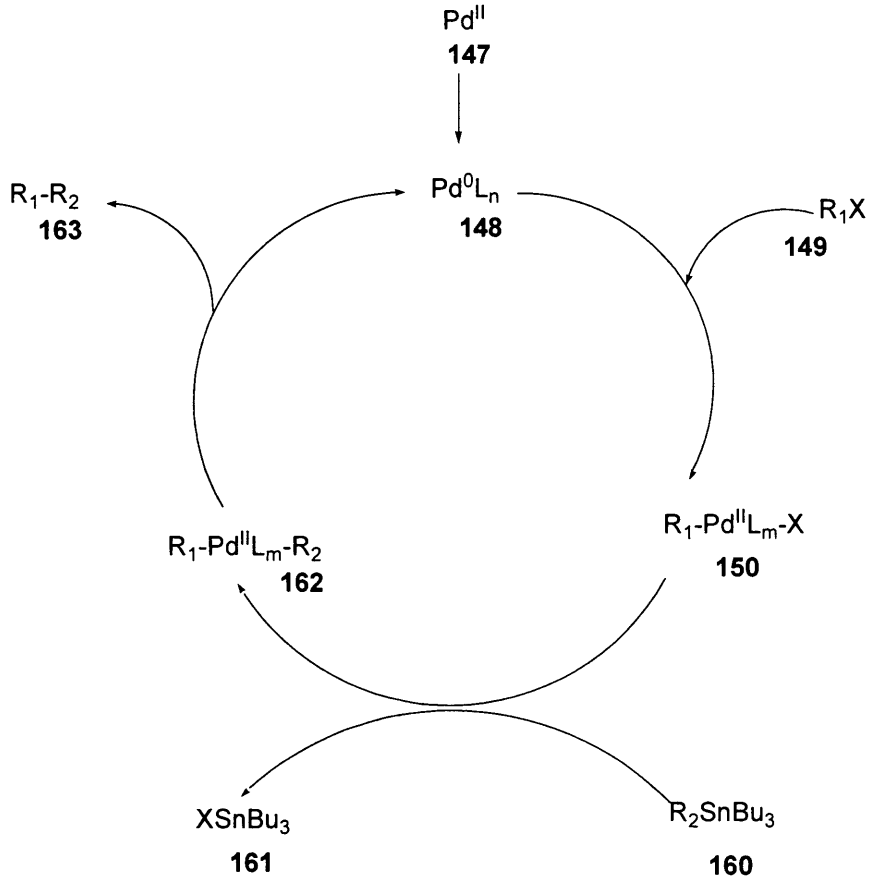
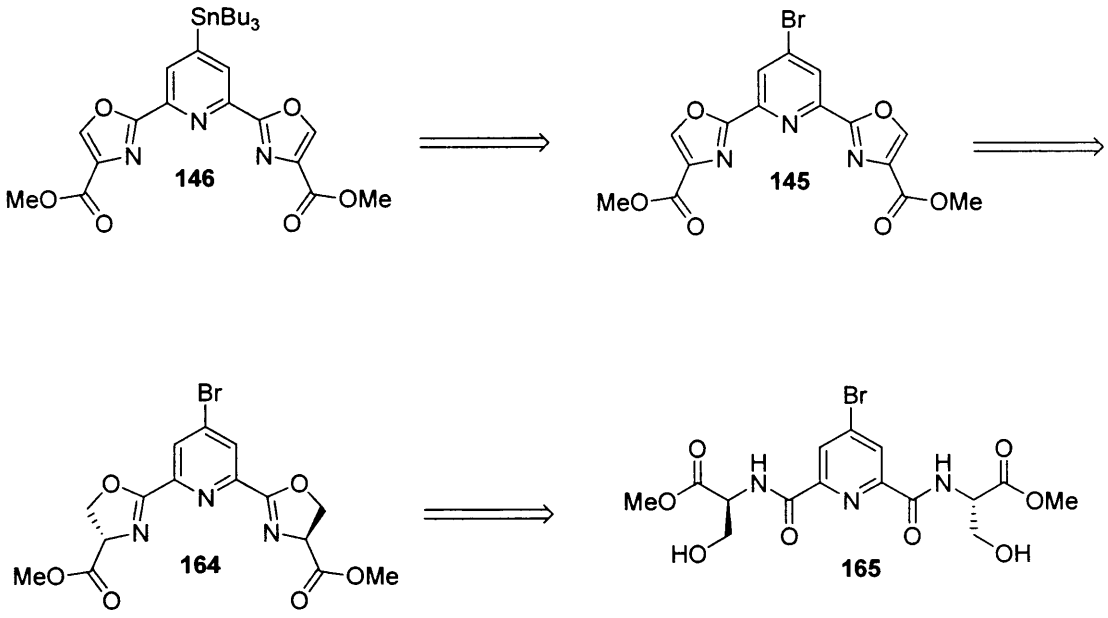


Figure 36.

A Suzuki coupling[5,6] also follows a similar mechanism to the Stille coupling, except that a boronic acid is used as the ligand in place of a stannane.

The stannane **146** can be synthesised from a stannylation reaction involving the bromopyridine **145** using dichlorobis(triphenylphosphine)palladium (II) and bis (tributyltin) heated to high temperatures (scheme 50).

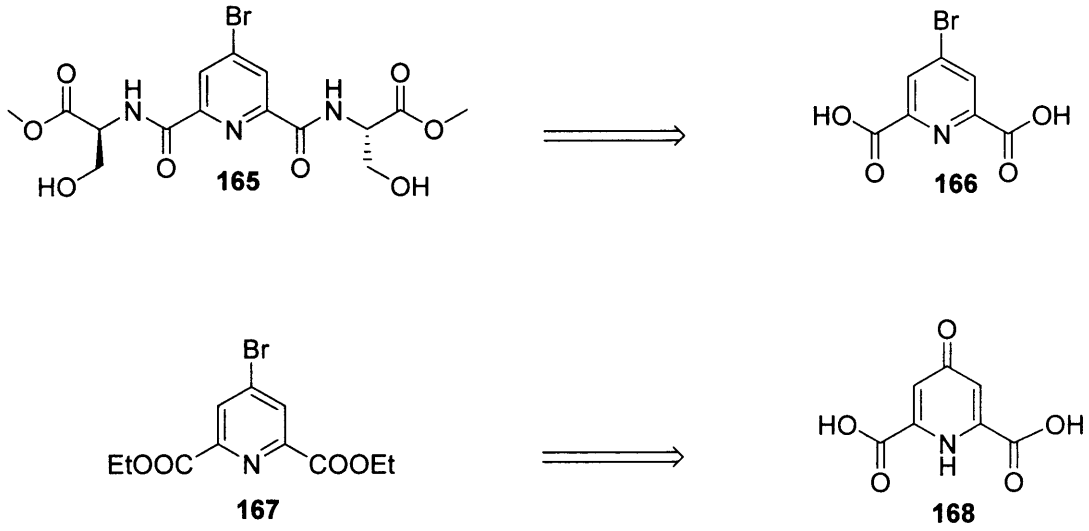


Scheme 50

The oxazolyl species **145** (scheme 50) can be obtained via a dehydrogenation reaction using DBU and bromotrichloromethane in dichloromethane following the Williams-Wipf procedure.[7,8]

The oxazoline species **164** (scheme 50) can be formed from by the cyclisation of the di amide **165**. The cyclisation process can be carried out by the use of DAST and potassium carbonate in dichloromethane.[8]

A double acylation of L-serine methyl ester with the pyridine diacid species **166** should afford the diamide **165** (scheme 51).[9]

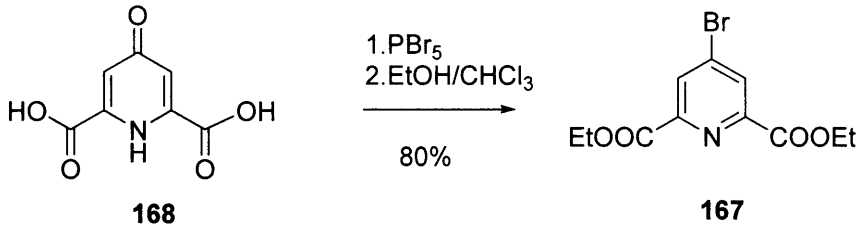


Scheme 51

The bromopyridine **167** has been obtained from chelidamic acid **168** using a procedure described by Takalo *et al* [10] (scheme 51).

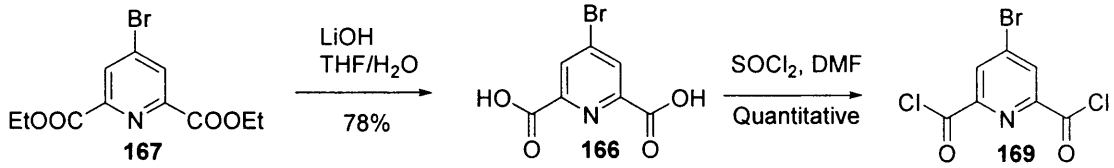
3. 3 Approaches to the synthesis of the tetraoxazolyl bipyridyl 144

The route required in the preparation of the bromopyridine **167** from chelidamic acid **168** by the treatment with neat phosphorus pentabromide. The reactive acid bromide went on to form the diethylester **167** after the addition of ethanol (scheme 52). The literature states that a 69% yield is obtained after a recrystallisation; fortunately a yield of 80% was obtained after column chromatography (using neat ethyl acetate).



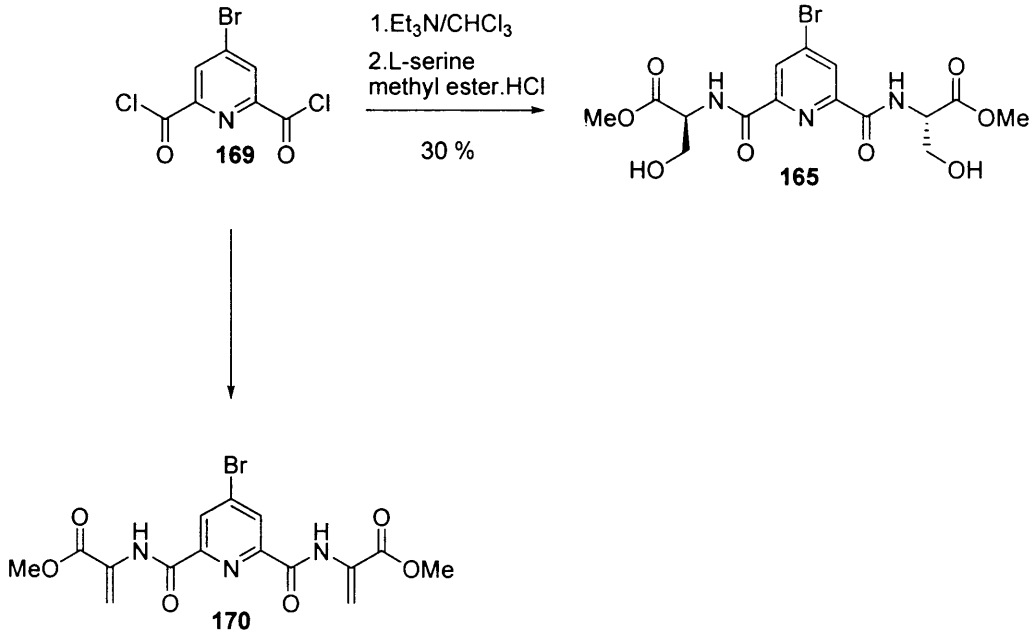
Scheme 52

The diacid **166** was obtained by hydrolysis reaction with lithium hydroxide in a 1:5 mixture of water and THF (scheme 53). After 3 hours, the mixture was acidified to pH 1. The solid material was difficult to handle due to coagulation but with gentle sonication the diacid **166** was obtained. Acid **166** was extremely insoluble in most solvents, preventing diamide **165** from being obtained. Hence, the diacid chloride **169** was prepared from the diacid **166** using thionyl chloride and two drops of DMF.



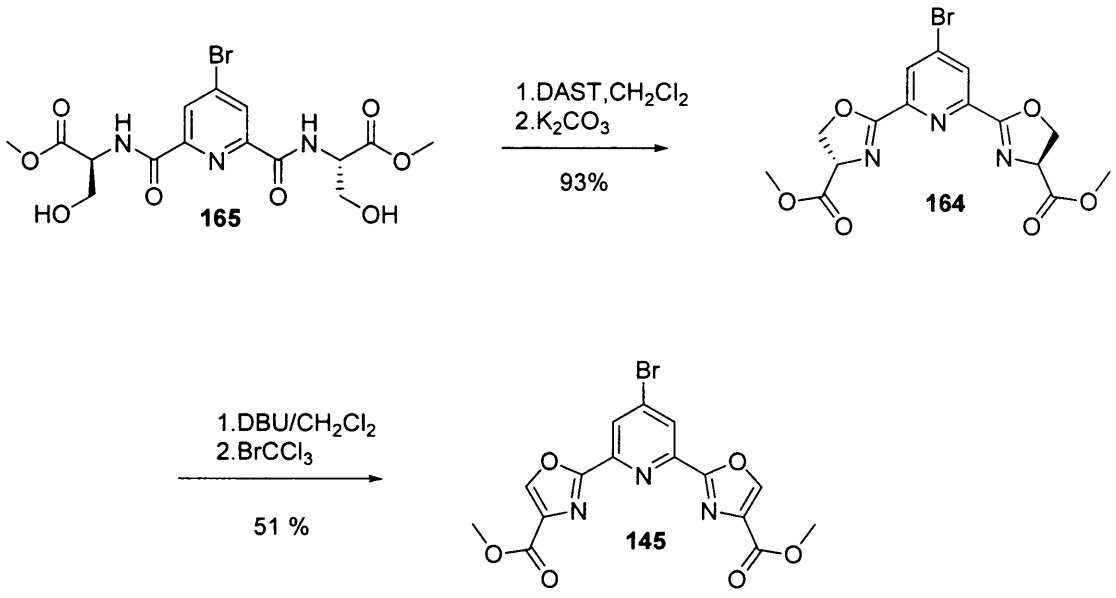
Scheme 53

The next step involved the amination of diacid chloride **16** a with L-serine methyl ester hydrochloride. However, the reaction proceeded in low yield. Small amounts of thionyl chloride may have been present that may have reacted with the free alcohol to form the eliminated product **170**, since many spots were found on the tlc plate (scheme 54).



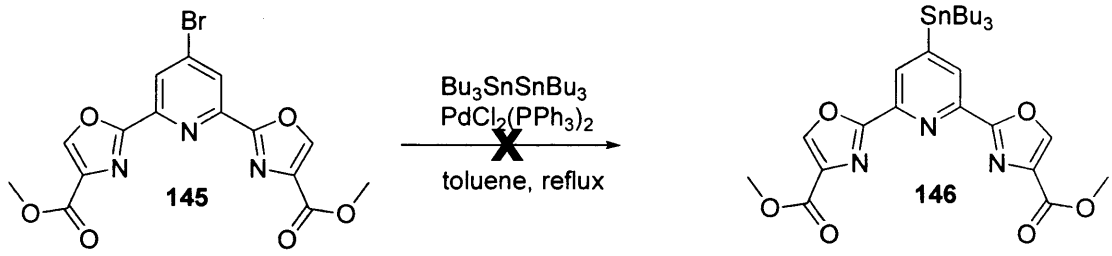
Scheme 54

The next step in the synthesis was the obtaining of the bisoxazoline **164** from the bisamidopyridine **165**. The reaction involved the reagents DAST and potassium carbonate at -78 °C. This reaction was very high yielding, 93% of the bisoxazoline **164** and with no detectable side-product. The ^{1}H NMR spectrum showed the characteristic multiplet at δ 4.70 for the oxazoline hydrogen atom.[11, 12] The next step involved the dehydrogenation of the bisoxazoline **164** using DBU and bromotrichloromethane. This reaction proceeded in satisfactory yield; ^{1}H NMR showed the absence of the multiplet found for **164,** but the presence of new oxazole peaks confirming that the bisoxazole **145** had been formed (scheme 55).



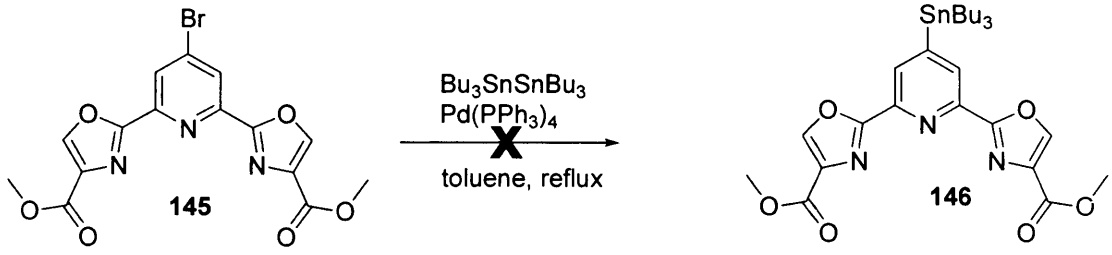
Scheme 55

The core bromopyridyl fragment **145** was successfully obtained. A similar procedure to that of Pryor *et al* [9] was carried out to form the stannane **146** using dichlorobis(triphenylphosphine)palladium (II) and bis (tributyltin). After heating at reflux for two hours, tlc of the reaction indicated many compounds had formed. Each spot was isolated and identified although none of the spots indicated that **146** had been obtained.



Scheme 56

Since dichlorobis(triphenylphosphine)palladium (II) had not been successful, the catalyst was replaced by palladium tetrakis(triphenylphosphine). However, after heating in toluene at reflux for two hours many side products had formed, and this did not lead to a successful outcome.

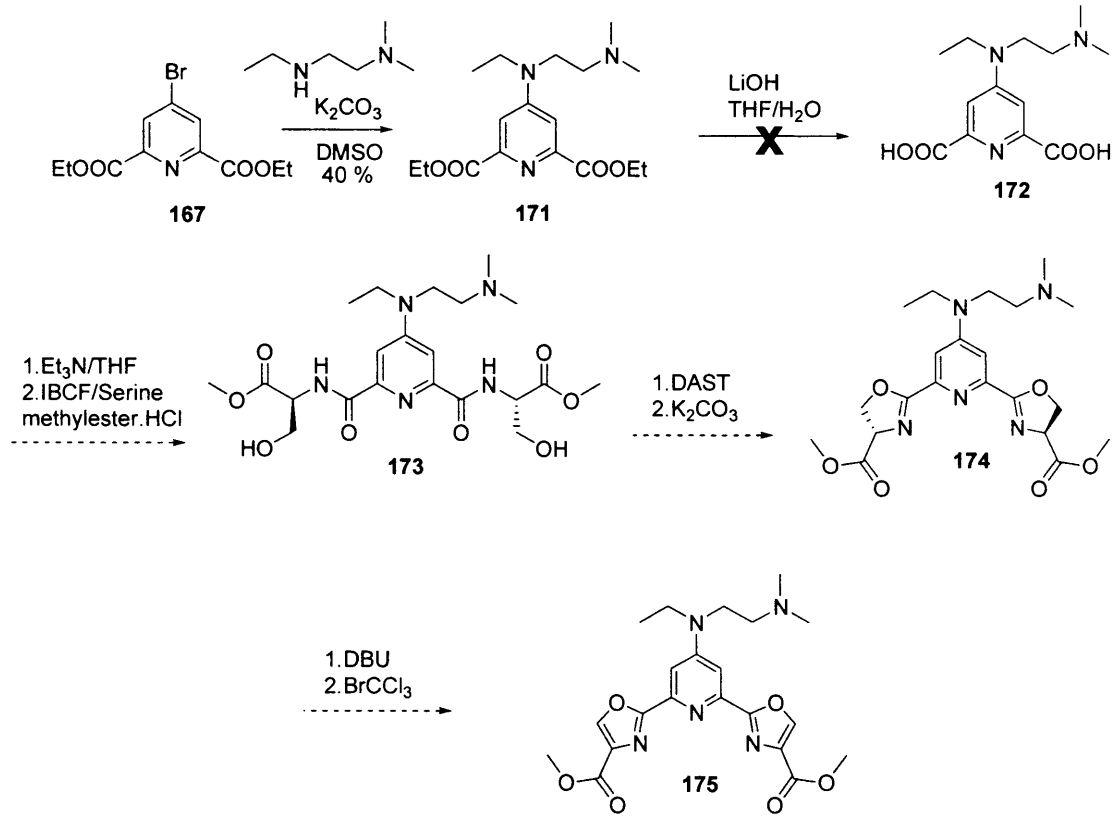


Scheme 57

The failure to obtain stannane **146** meant that no progress could be made beyond bromopyridine **145** and the proposed route had to be abandoned.

3.4 Approaches to the synthesis of bisoxazolylpyridine 175

Another goal was to obtain the bisoxazole **175**, in which the bromo group of bisoxazole **145** has been displaced by a secondary amine. The sequence began by reacting the bromide **167** with N^1-ethyl-N^2, N^2-dimethylethane-1,2-diamine in the presence of potassium carbonate and DMSO to form the tertiary amine **171**. The reaction was successful, although the yield was quite low; many side-products have formed. The next step involved the hydrolysis of the ester to form the diacid **172**. The tlc confirmed the reaction went to completion; however, the work-up proved very difficult. It was found that acidification using 3 M HCl protonated the amine which made it extremely difficult to dissolve in any common solvents. It was decided that the lithium salt of **172** would be used to form the bisamide **173**, thereby eliminating the work-up step. However, this reaction was also unsuccessful; tlc showed no product was forming. Owing to lack of time the route to **175** was abandoned.



Scheme 58

References

(1) Milstein, D.; Stille, J. K. *J. Am. Chem. Soc.* **1978**, *100*, 3636-3638.

(2) Casado, A. L.; Espinet, P. *J. Am. Chem. Soc.* **1998**, *120*, 8978-8985.

(3) Stille, J. K. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508-523.

(4) Casado, A. L.; Espinet, P. *Organometallics.* **1998**, *17*, 954-959.

(5) Miyaura, N.; Yamada, K.; Suzuki, A. *Tetrahedron Lett.* **1979**, 3437-3440.

(6) Miyaura, N.; Suzuki, A. *J. Chem. Soc., Chem. Commun.* **1979**, 866-867.

(7) Williams, D. R.; Lowder, P. D.; Gu, Y. G.; Brooks, D. A. *Tetrahedron Lett.* **1997**, *38*, 331-334.

(8) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.

(9) Pryor, K. E.; Shipps, G. W.; Skyler, D. A.; Rebek, Jr. *Tetrahedron* **1998**, *54*, 4107-4124.

(10) Takalo, H.; Kankare, J. *J. Acta. Chem. Scand.* **1987**, *41*, 219-221.

(11) Iwasa, S.; Nakamura, H.; Nishiyama, H. *Heterocycles* **2000**, *52*, 939-944.

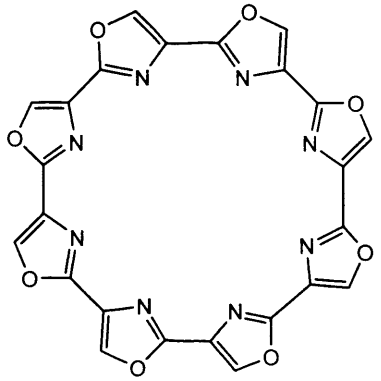
(12) Endoh, N.; Tsuboi, K.; Kim, R.; Yonezawa, Y.; Shin, C. *Heterocycles* **2003**, *60*, 1567-1572.

Chapter 4

4.0 Approaches to the synthesis of cyclooctaoxazole 176

4.1 Introduction

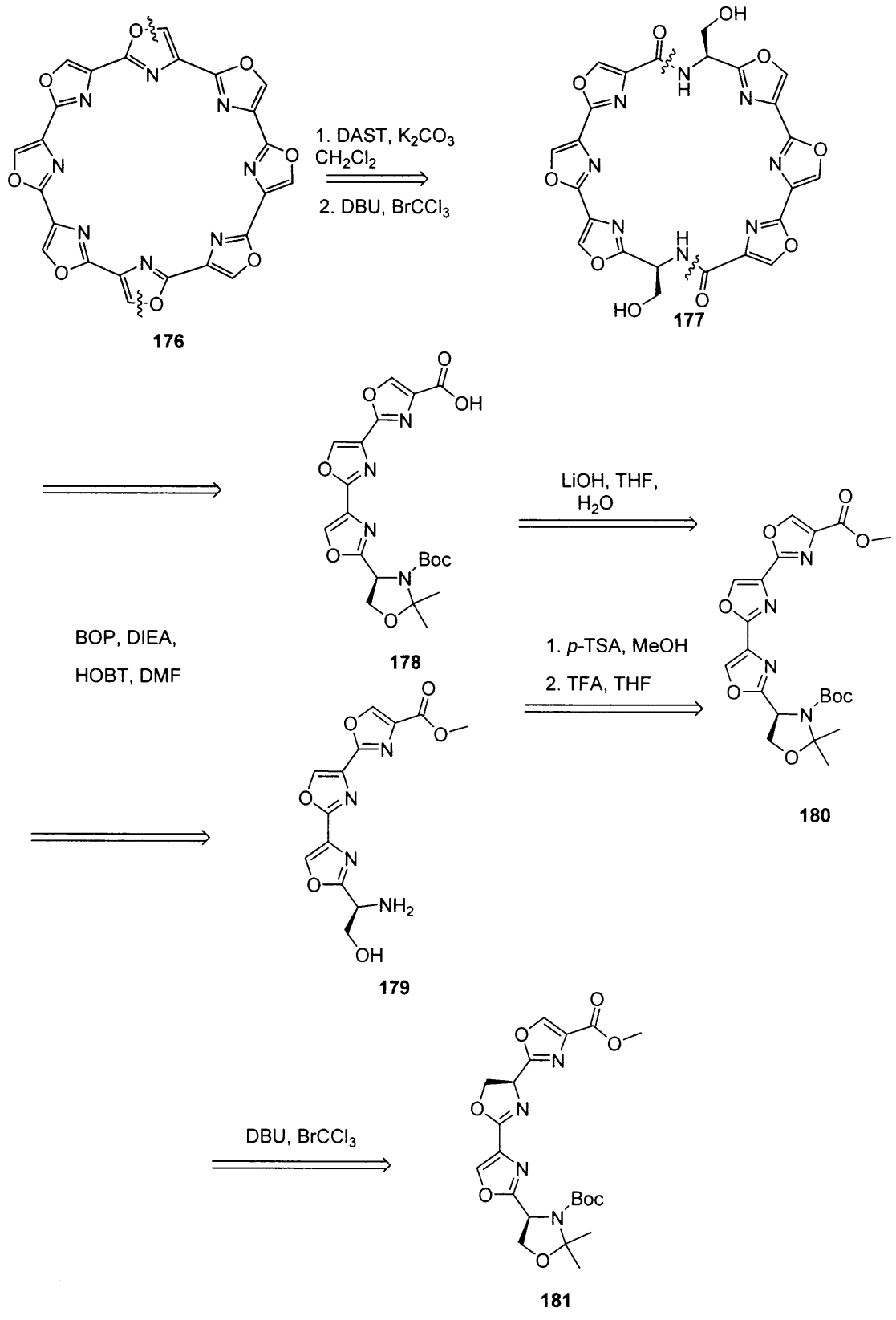
The aim of this section was to synthesise an analogue of telomestatin such as the symmetrical polyoxazole system **176.** Such cyclic systems would be evaluated for specific binding to telomeric DNA G–quadruplexes and inhibitors of the human enzyme telomerase. The structure resembles telomestatin being planar, electron deficient and containing multiple oxazole rings, as well as containing an internal planar location of eight sp^2-hybridised nitrogen atoms.



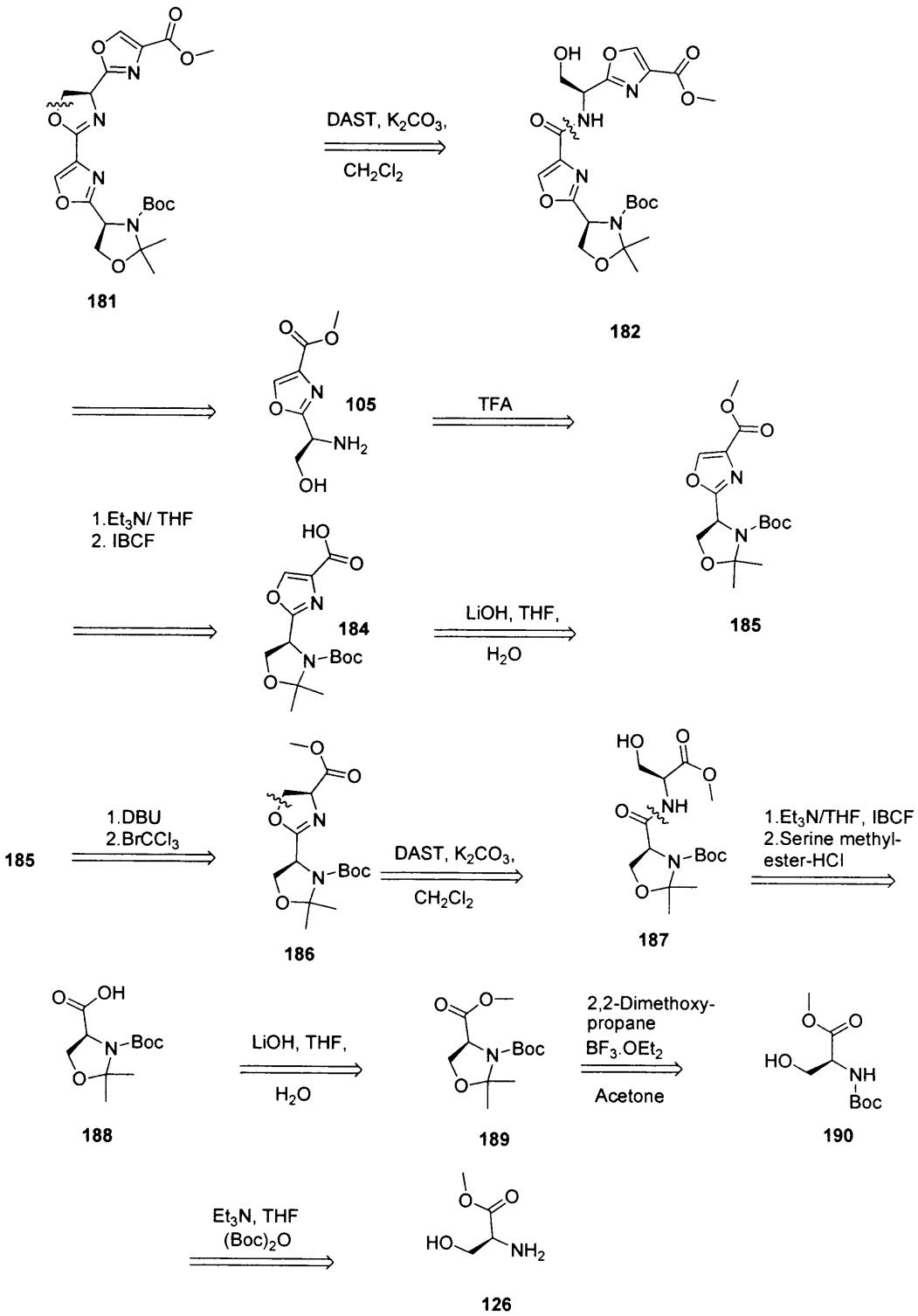
176

Figure 37. An important cyclic polyoxazole target

From a retrosynthetic viewpoint the synthesis of **176** would require the following compounds:



176 **177** **178** **179** **180** **181**

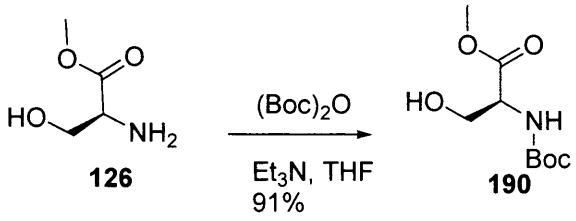

DAST, K_2CO_3,
CH_2Cl_2
181
182
105
TFA
1.Et_3N/ THF
2. IBCF
184
LiOH, THF,
H_2O
185
1.DBU
2.$BrCCl_3$
185
DAST, K_2CO_3,
CH_2Cl_2
186
187
1.Et_3N/THF, IBCF
2.Serine methyl-ester-HCl
LiOH, THF,
H_2O
188
189
2,2-Dimethoxy-propane
$BF_3.OEt_2$
Acetone
190
Et_3N, THF
$(Boc)_2O$
126

Scheme 59

Results and discussion

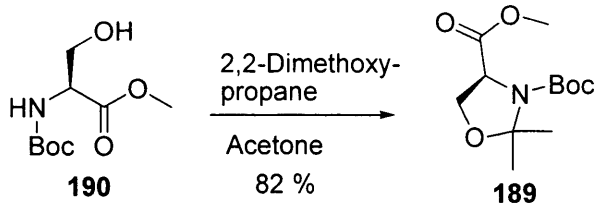
4.2 The first approach: condensation of two trisoxazole units using Boc as the protecting group on nitrogen

The attempted synthesis of **176** followed the reverse of the retrosynthetic strategy shown in scheme 60. The route began with *N*-protection of serine methyl ester hydrochloride using Boc anhydride and triethylamine in THF giving carbamate **190** as a colourless oil in 91 % yield (scheme 60).[1]



Scheme 60

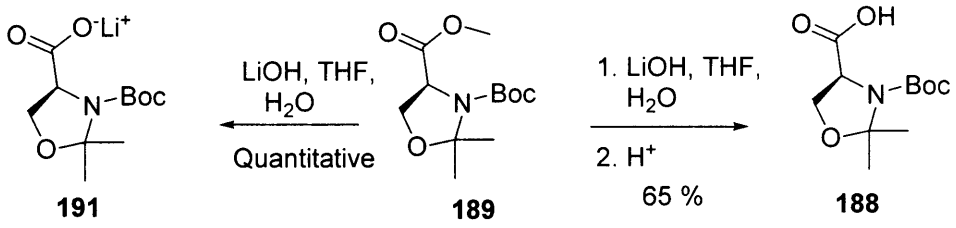
The next stage of the synthesis required the protection of the amino alcohol unit as the *gem*-dimethyl oxazolidine **189**, using 2,2 dimethoxypropane and boron trifluoride-diethyletherate in acetone (scheme 61). Although this protection step afforded several products (as shown by tlc), a slow elution on a silica column with petroleum ether: ethyl acetate (10: 0.5) afforded **189** in 82 %.[1-3]



Scheme 61

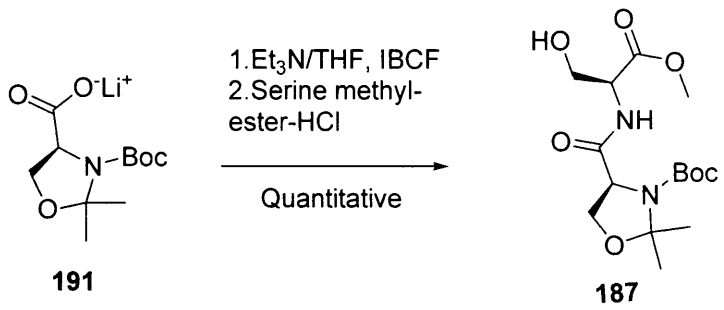
The methyl ester **189** was then hydrolysed to the acid **188** using one equivalent of lithium hydroxide in aqueous THF. After an acid work-up, acid **188** was isolated in 65% (scheme 62). The hydrolysis step was optimised by

evaporating the solvent of the reaction mixture to dryness to leave the free carboxylated oxazolidine **191** without any acidification. This procedure improved the yield greatly, affording the crude quantitatively.



Scheme 62

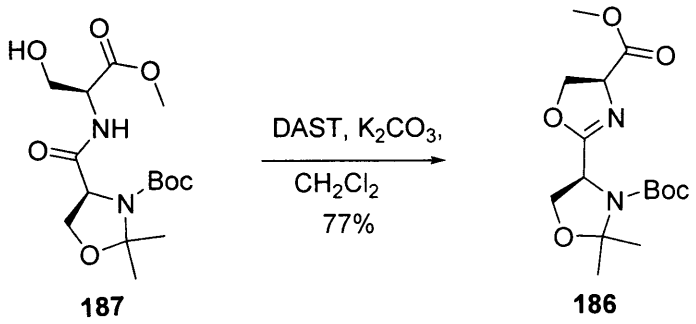
The carboxylate **191** was then acylated with L-serine methyl ester using isobutyl chloroformate as the coupling agent (scheme 63). The reaction was allowed two hours to complete; after work-up, the amide **187** was isolated as a colourless oil. However, obtaining the amide **187** pure was undesirable in terms of yield, since isolation required column chromatography (in neat ethyl acetate) and the amide **187** adhered to silica gel, giving an isolated yield of only 40%, even when 20% methanol was used for elution.



Scheme 63

A trial was carried out to see how an impure sample would react in the subsequent step; 200 mg of crude amide **187** was reacted with one equivalent diethylamino sulfur trifluoride to form the corresponding oxazoline **186** (scheme 64). Results showed that an unpurified sample of amide **187**, did not affect the

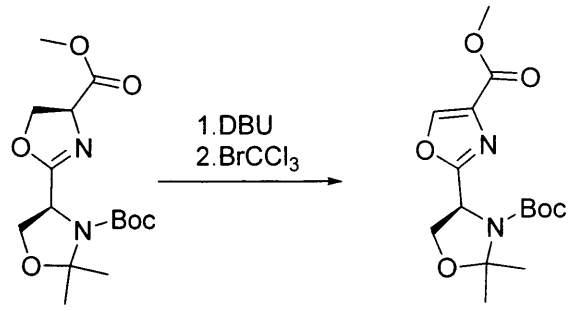
yield of oxazoline **186**. The method was successfully repeated on a 5 g scale. However, it was found that if the reaction was repeated on a scale greater than 5 g the yield was dramatically decreased by up to 20%.[4,5]



187 **186**

Scheme 64

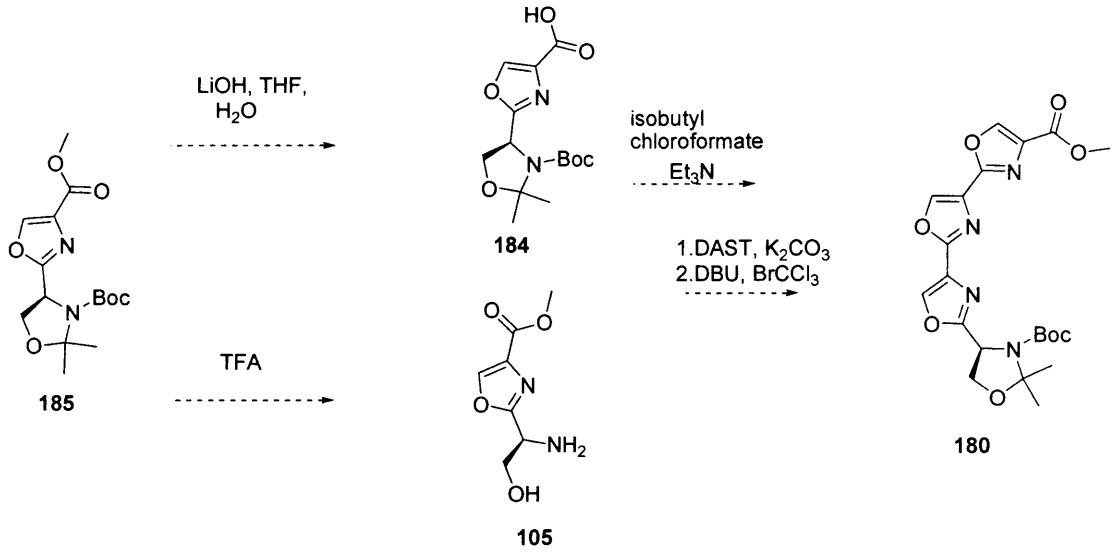
The oxazoline **186** was successfully converted into the oxazole **185** in quantitative yield using DBU and bromotrichloromethane (scheme 65).[1] Compounds **186** and **185** were each purified by column chromatography without complications.



186 **185**

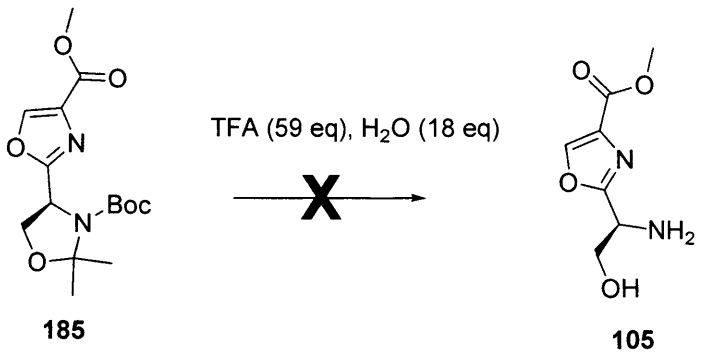
Scheme 65

Corresponding to the retrosynthesis of compound **176** in scheme 59, the synthetic sequence was successful up to the oxazole step and with no major problems. The common intermediate in the sequence was the oxazole **185** which was to be converted in a convergent manner into the acid **184** and amino alcohol **105** (scheme 66). These compounds would then be coupled together to form the trisoxazole precursor **180**.[6]



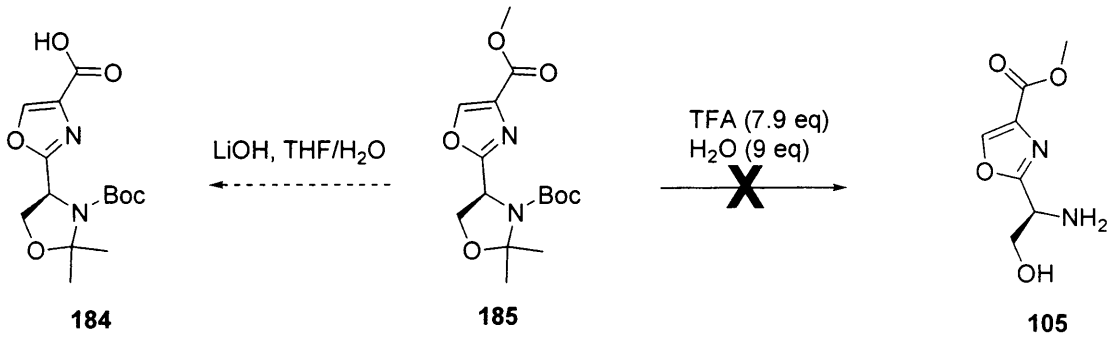
Scheme 66

Several methods of deprotection to give the amino alcohol **105** were tried but proved quite troublesome. Firstly, a modified procedure[1] was used whereby trifluoroacetic acid (TFA) (59 eq) in water (18 eq) was used to react with **185** and then left to stir at room temperature for 14 hours. The mixture was later basified to pH 8 using saturated aqueous sodium hydrogen carbonate. Tlc results showed the completion of reaction (as in scheme 67), as a baseline spot appeared and no protected oxazole **185** was found; however, ^{1}H NMR spectroscopy of the crude reaction mixture showed that the Boc group was still present. The result seemed to indicate that the reaction had not gone to completion; therefore further modifications were made.



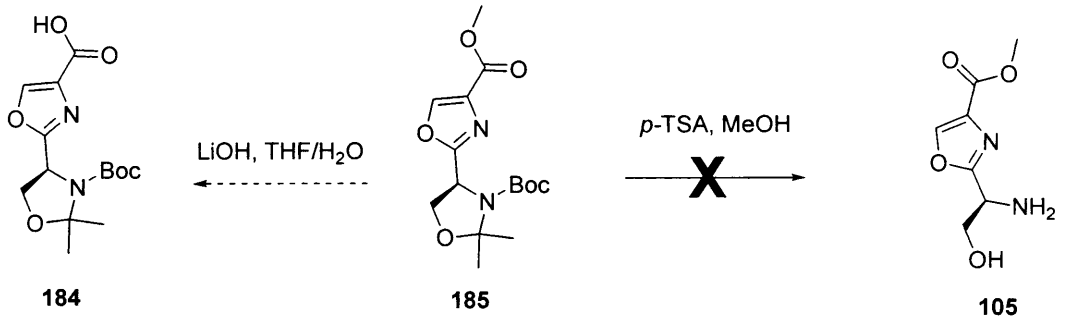
Scheme 67

In a second attempt, a different procedure was followed, using 7.9 equivalents of TFA and 9 equivalents of water. The mixture was heated to 50 °C and after 10 minutes no starting material was found by tlc. The reaction mixture was left for a further 2 hours at 50 °C (scheme 68), after which the solvent was evaporated to dryness and the residue basified to pH 8. However, extraction with ethyl acetate gave a poor yield. It was thought that the product may be very polar and remain in the aqueous aqueous layer, according the aqueous layer, was evaporated by azeotropic removal of water using toluene, giving a high yield of brown oil. However, ^{1}H NMR spectroscopy again showed only partial removal of Boc group.



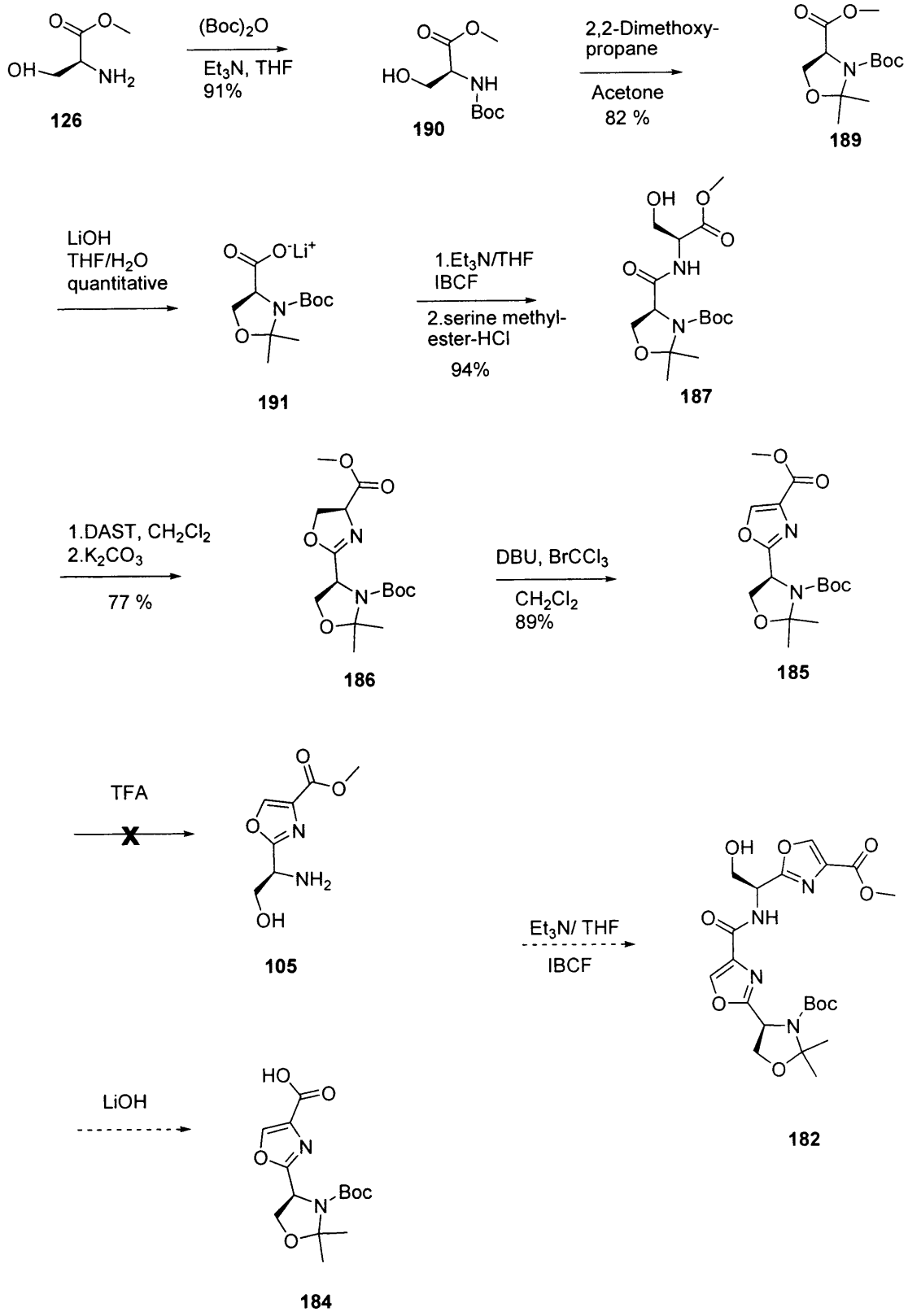
Scheme 68

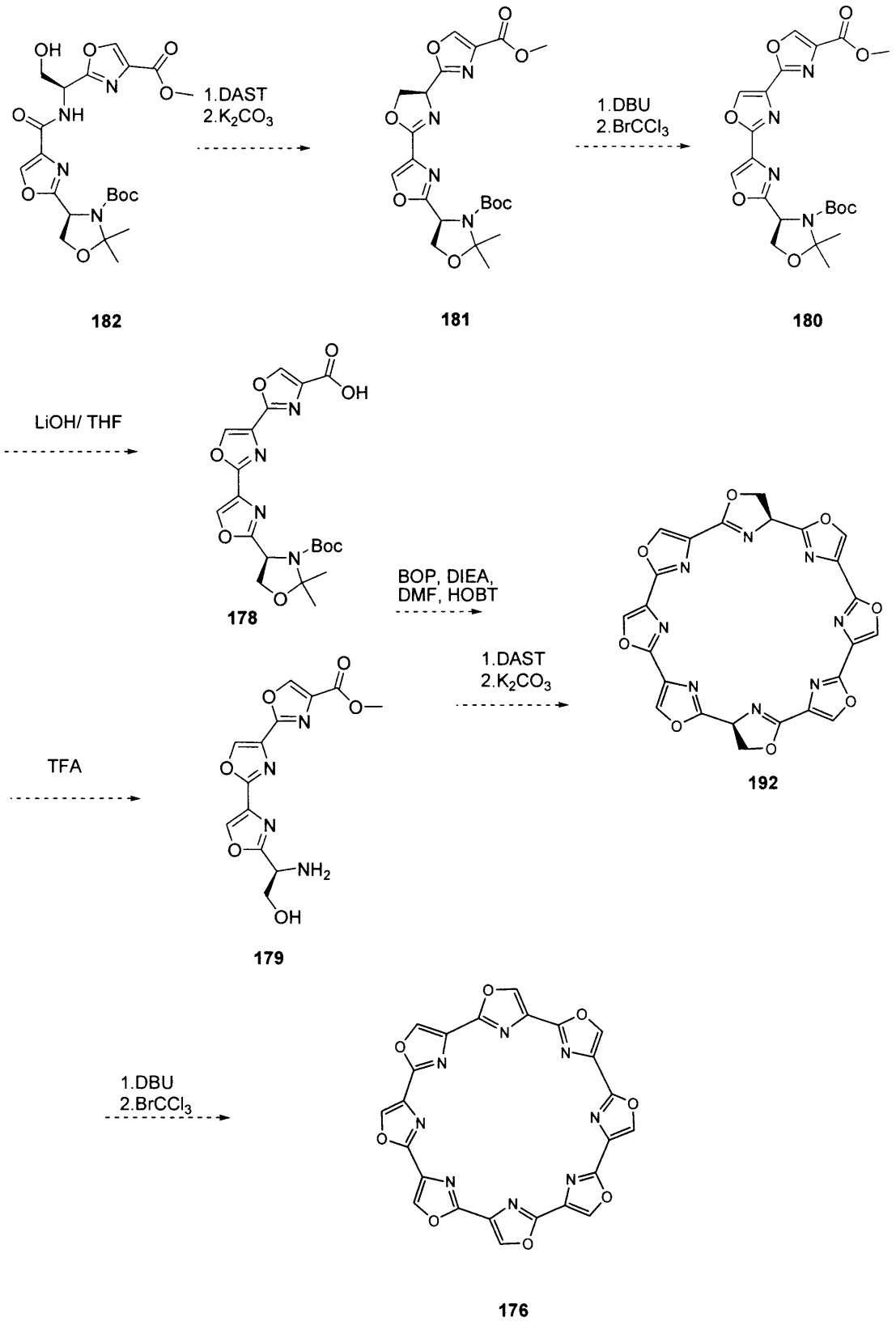
Using TFA and heating **185** to extreme temperatures was not desirable owing to the sensitive and reactive groups present in the product **105**. It was found that TFA transformed the oxazole **185** from an oil into a gelatinous material which proved very difficult to handle as it did not dissolve in common organic solvents or even in water. The next attempt was to deprotect the oxazole **185** using *p*-TSA (1 equivalent) in methanol as it was a milder acid (scheme 69). The tlc showed that a new product had formed, and that no starting material remained. However, the product was isolated and shown not to be **105**. Since amino alcohol **105** could not be obtained by any of several methods used, hydrolysis of ester **185** to the acid **184** was not attempted because acid **184** could not be used in the synthesis.



Scheme 69

In conclusion, the first approach to octaoxazole **176** resulted in oxazole **185** (scheme 70). However it proved very difficult to prepare the amino alcohol **105** owing to incompleteness of the reaction and losses during manipulation. The route was then discontinued and an alternative one was investigated.


126
(Boc)2O
Et3N, THF
91%
190
2,2-Dimethoxy-
propane
Acetone
82 %
189
LiOH
THF/H2O
quantitative
191
1.Et3N/THF
IBCF
2.serine methyl-
ester-HCl
94%
187
1.DAST, CH2Cl2
2.K2CO3
77 %
186
DBU, BrCCl3
CH2Cl2
89%
185
TFA
105
Et3N/ THF
IBCF
182
LiOH
184

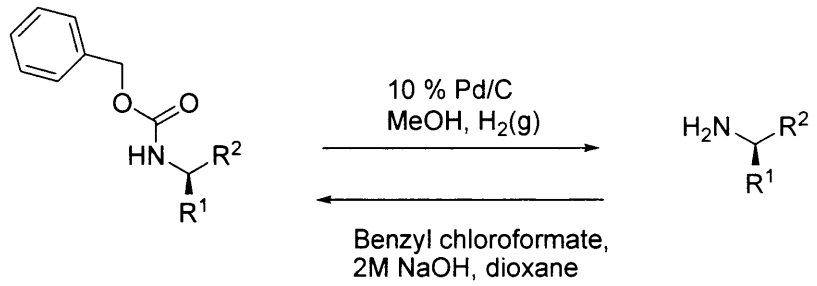


182 **181** **180** **178** **192** **179** **176**

Scheme 70 The projected first route to the cyclic oxazole **176**

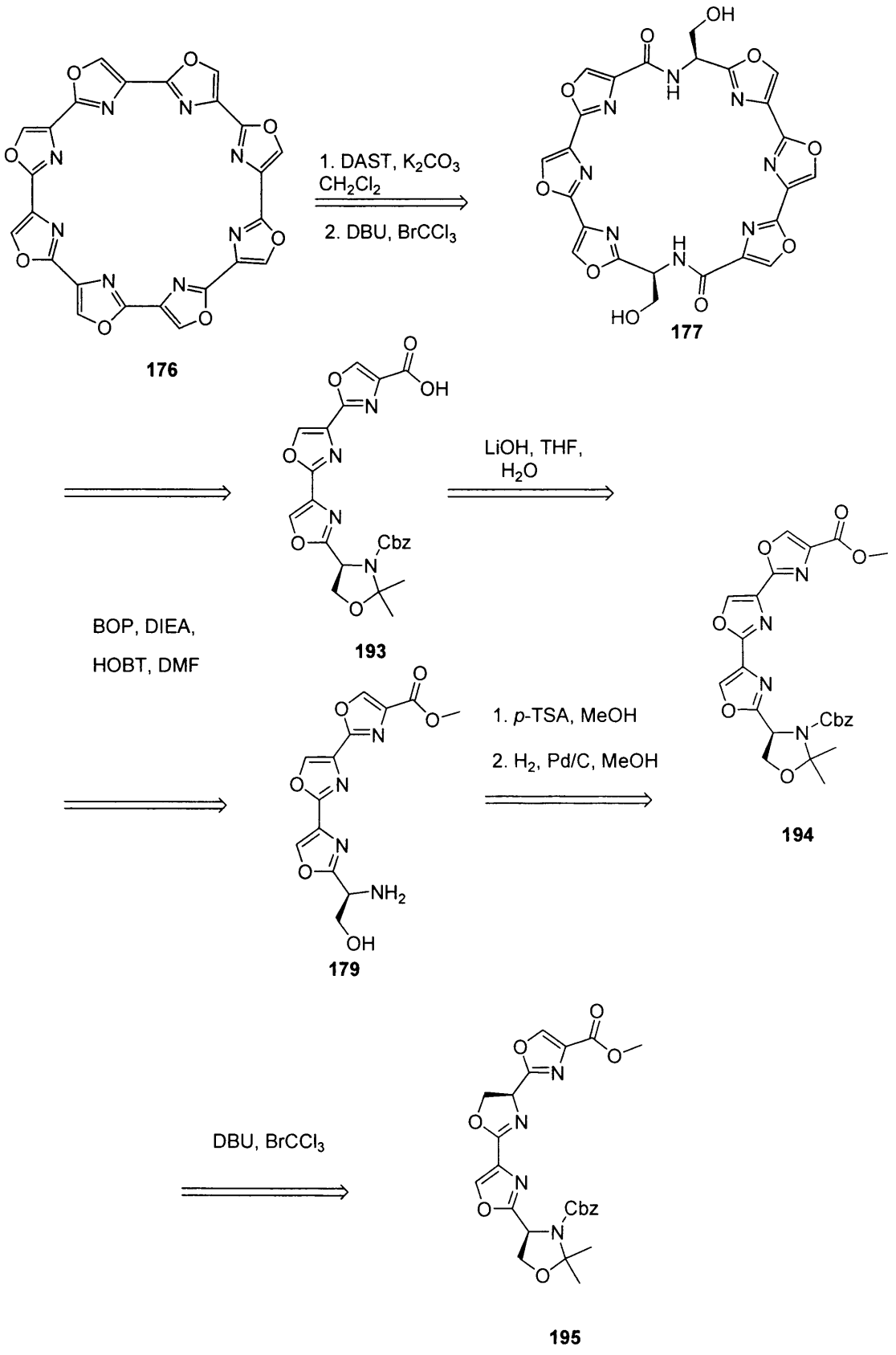
4. 3 A second approach: condensation of two trisoxazole units using Cbz as the protecting group

The first approach to macrocycle **176** was unsuccessful owing to problems with deprotection of the Boc group. Hence, an alternative route was required to resolve the problem. In the second approach, the Cbz protecting group was chosen because deprotection by hydrogenation is conducted under neutral conditions, which is highly desirable since acids such as TFA had been found to convert **185** into a gelatinous material that was difficult to manipulate. Deprotection and protection are thus accomplished as in scheme 71.

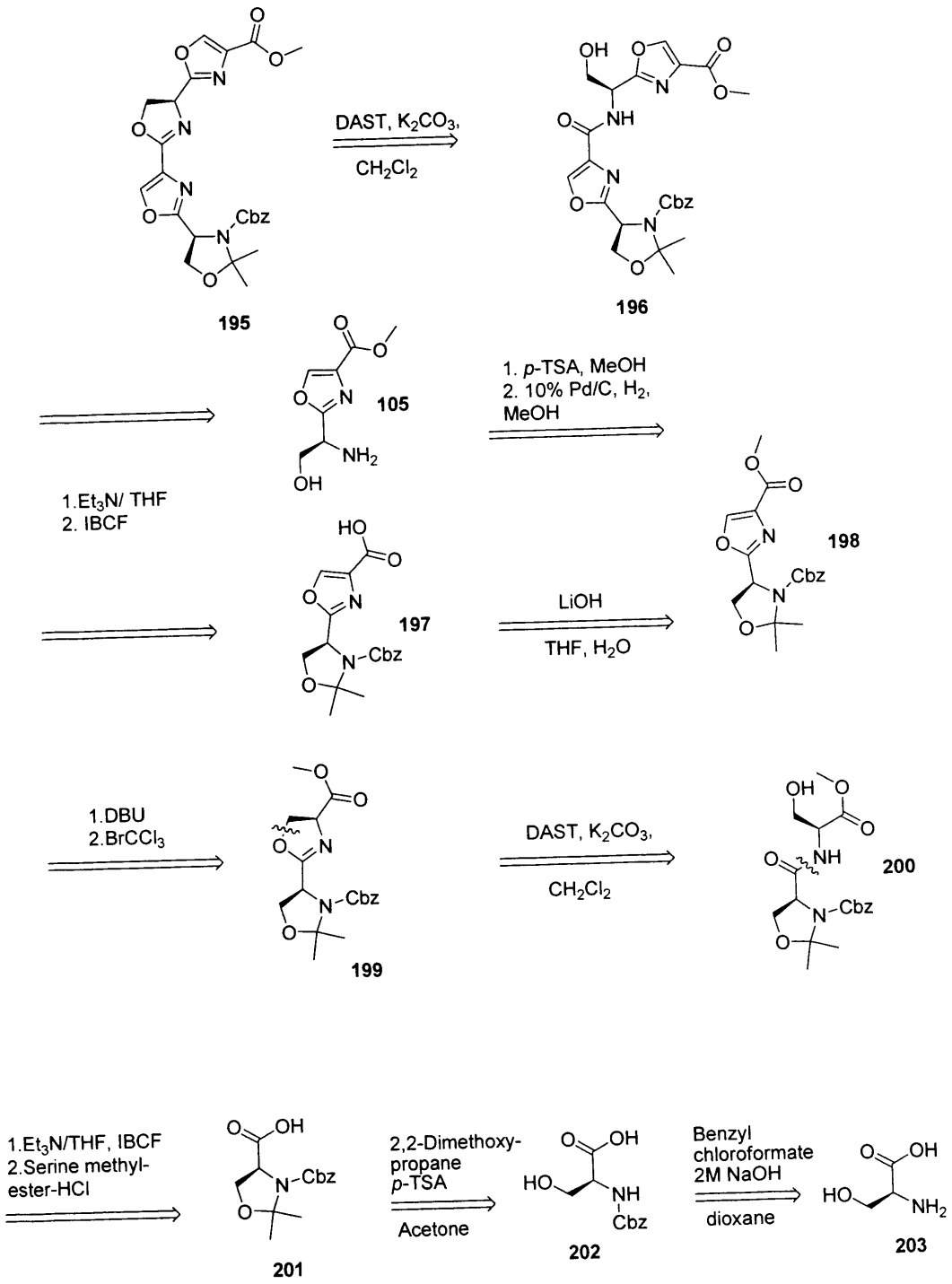


Scheme 71

The revised retrosynthesis is shown below:

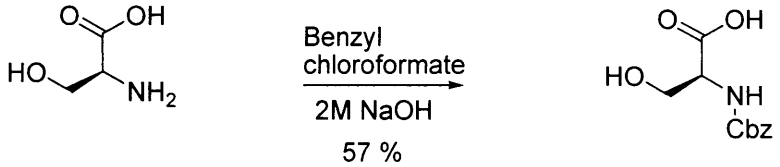


176 **177** **193** **179** **194** **195**


DAST, K_2CO_3,
CH_2Cl_2
195
196
105
1. p-TSA, MeOH
2. 10% Pd/C, H_2,
MeOH
1.Et_3N/ THF
2. IBCF
197
198
LiOH
THF, H_2O
1.DBU
2.$BrCCl_3$
199
DAST, K_2CO_3,
CH_2Cl_2
200
1.Et_3N/THF, IBCF
2.Serine methyl-
ester-HCl
201
2,2-Dimethoxy-
propane
p-TSA
Acetone
202
Benzyl
chloroformate
2M NaOH
dioxane
203

Scheme 72

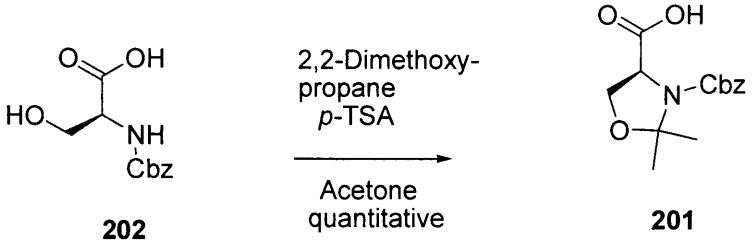
Obtaining acid **202** from **203** involved the stirring of benzyl chloroformate, 2 M NaOH and 1,4-dioxane for 48 hours at room temperature to give **202** in 57% yield (scheme 73). However, the purification of the reaction was quite difficult because quantities of benzyl chloroformate appeared to contaminate the product even after carrying out column chromatography; moreover, benzyl chloroformate is difficult to observe on tlc plates, even with staining. However, a very slow elution with 1% ethyl acetate/ petroleum ether was used; once isolated, acid **202**[7] was a bright white solid with a sharp melting point of 119-120 °C.



203 **202**

Scheme 73

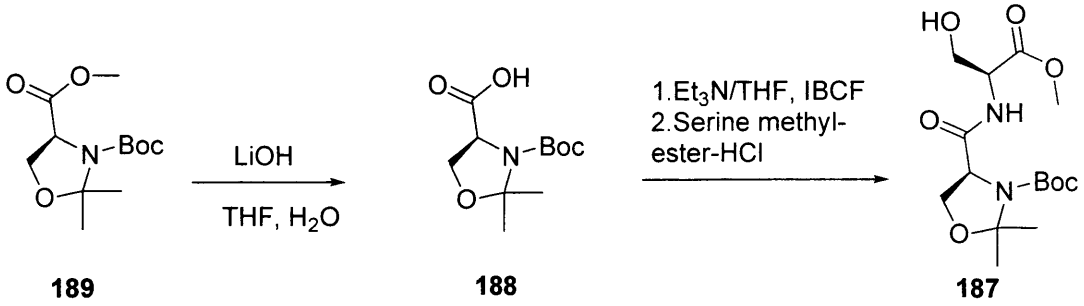
The second step in the synthesis of **176** required the formation of the oxazolidine **201**. Initially, boron trifluoride diethyl etherate was used as the catalyst (in the presence of 2,2-dimethoxypropane) to aid the formation of the oxazolidine **201**, it had been in the Boc protection (first approach) to obtain **189**, but unfortunately the reaction was unsuccessful. An alternative attempt involved reacting **202** with *p*-TSA, 2,2-dimethoxypropane in acetone at 45 °C for 12 hours (scheme 74). Using *p*-TSA was successful; the reaction proceeded to completion and gave no side-products. It was also found that if the temperature of the reaction was higher than 45 °C, the *gem*-dimethyl group was cleaved to give the free alcohol **202**. The same result also occurred when the reaction mixture was stirred for longer than 12 hours at 45 °C.



202 **201**

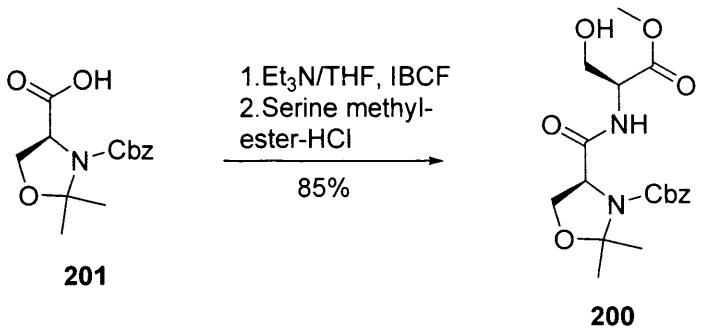
Scheme 74

The new route (scheme 72) to cyclooctaoxazole **176** seemed more efficient since the step involving the hydrolysis of the ester **189** (scheme 75) was avoided.[8]



Scheme 75

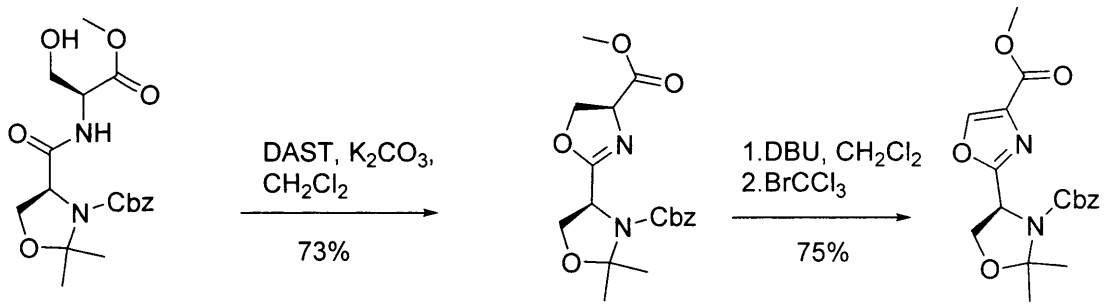
In the revised route, acid **201** was coupled with L-serine methyl ester hydrochloride using isobutyl chloroformate to give amide **200** (scheme 76). The reaction went to completion provided that an excess of L-serine methylester (1.6 eq) was used. However, the yield decreased from 85% to 40% when purified on silica gel. Therefore, in this step crude material **200** was not purified by column chromatography, but was used directly in the next step.[1]



Scheme 76

The amide **200** was then converted into the oxazoline **199** using DAST under anhydrous conditions at -78 °C in CH_2Cl_2. The reaction proceeded in 73% yield, and with no major side-products. However, oxazoline **199** was shown to be unstable, and if left for a few days appeared to revert to the amide **200** (scheme

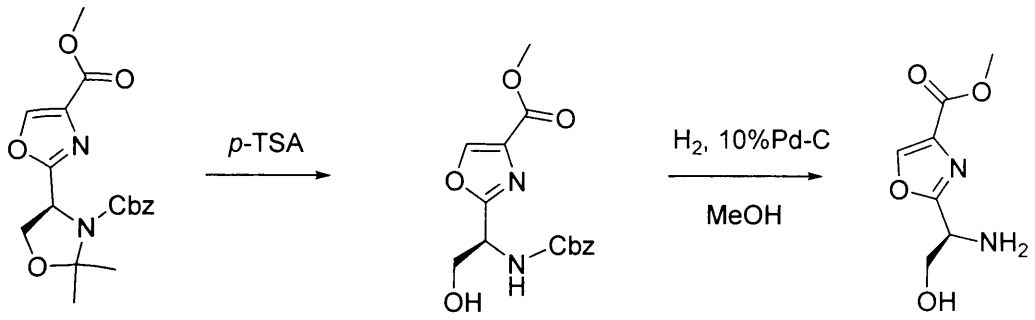
77). Accordingly, once **199** was formed, it was immediately converted into the oxazole **198** using DBU and bromotrichloromethane to give the desired oxazole in 75% yield.



200 **199** **198**

Scheme 77

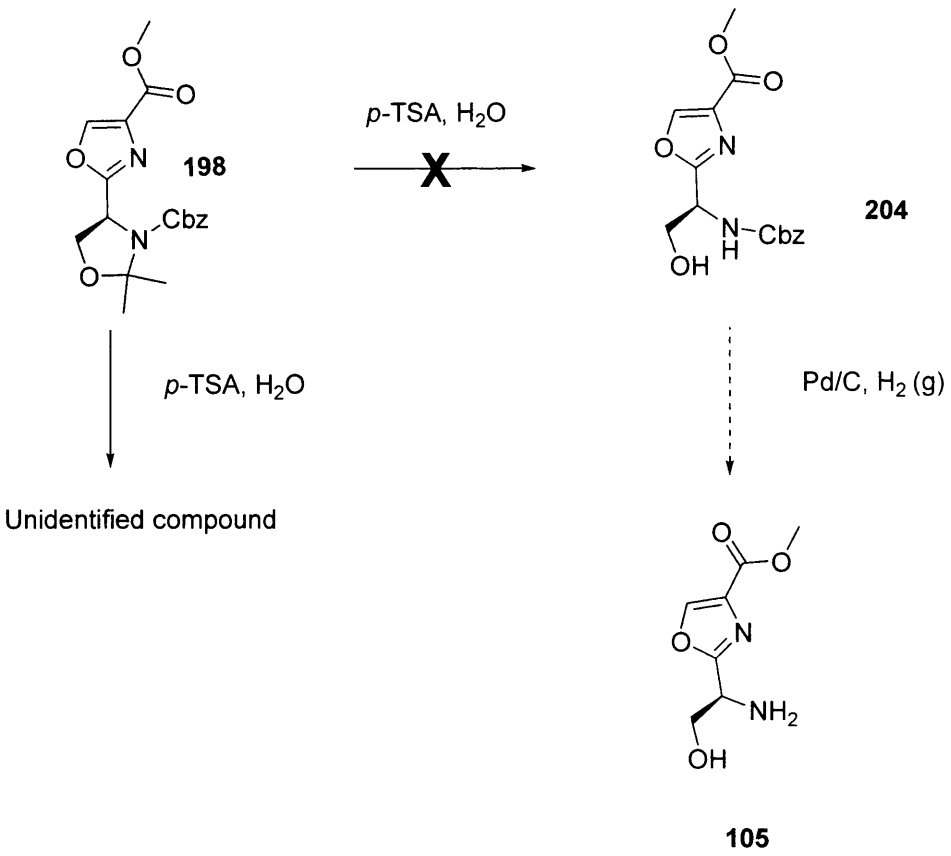
Deprotection of **198** was carried out using hydrogenation. It was thought that the free amine **105** would be difficult to manipulate owing to its insolubility, as previously found when TFA was used **185**; accordingly, the *gem*-dimethyl group was deprotected first using *p*-TSA, followed by hydrogenolysis to remove the Cbz group (scheme 78).[1,9]



198 **204** **105**

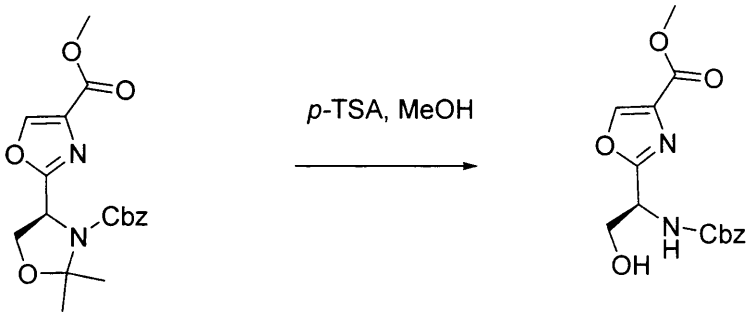
Scheme 78

The first attempt to deprotect **198** to give **204** was attempted using *p*-TSA and water. Tlc results indicated that the reaction was complete; however, the product formed (which stained red with anisaldehyde) was found to be some unidentifiable compound (scheme 79). ^{1}H NMR spectra showed all the major peaks required; however, the CH_2 and oxazole signals had shifted downfield.



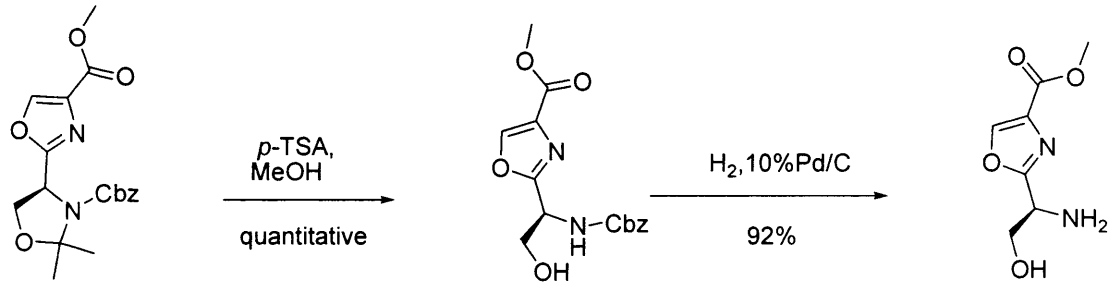
Scheme 79

Water was not used in the next attempt, but instead only one equivalent of *p*-TSA in methanol, when heated under reflux. The alcohol **204** was obtained in quantitative yield, and stained orange on silica impregnated with anisaldehyde (scheme 80). ^{1}H NMR and ^{13}C NMR data confirmed that the product **204** was synthesised as no aryl peaks were found. The reaction must not be left to stir for more than 2.5 hours under reflux as the product undergoes decomposition to give unidentified compound.



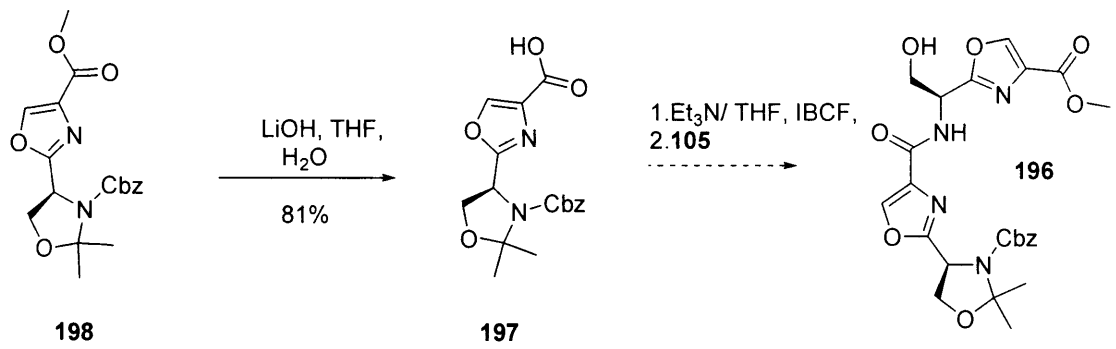
Scheme 80

Hydrogenolysis of the Cbz group in **204** was carried out using 10% Pd/C, suspended in methanol (scheme 81). The method proved successful as **105** was formed in the excellent yield 92%. The amino alcohol **105** did not require purification, since NMR spectroscopy showed the product to be free from any significant impurity. In this way, the deprotection step avoided the use of a strongly acidic medium or base work-up, factors which had proved so troublesome in the first approach to the amino alcohol **105**.



Scheme 81

The second fragment required for synthesis of precursor **196** of the trisoxazole was the oxazole carboxylic acid **197.**[1] Therefore, a hydrolysis reaction was carried out on the ester **198** using lithium hydroxide and water scheme 82. The reaction was high yielding but required heating at 89 °C for 17 hours, since at lower temperatures the reaction was incomplete.

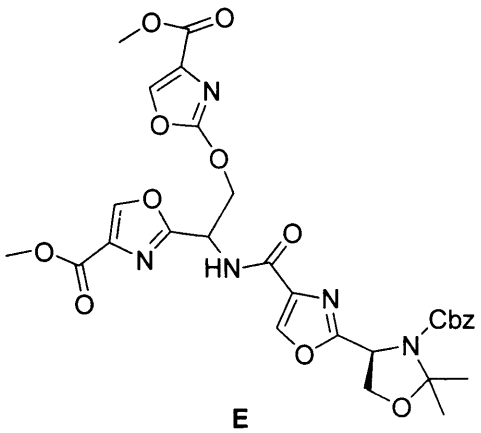


Scheme 82

The purification of acid **197** was carried out by evaporating the THF and extracting the remaining aqueous mixture with ethyl acetate. The organic layer was then discarded as it contained all the organic impurities. The aqueous layer which contained the carboxylate was acidified to pH 1 to liberate the pure acid **197** without the need for column chromatography.

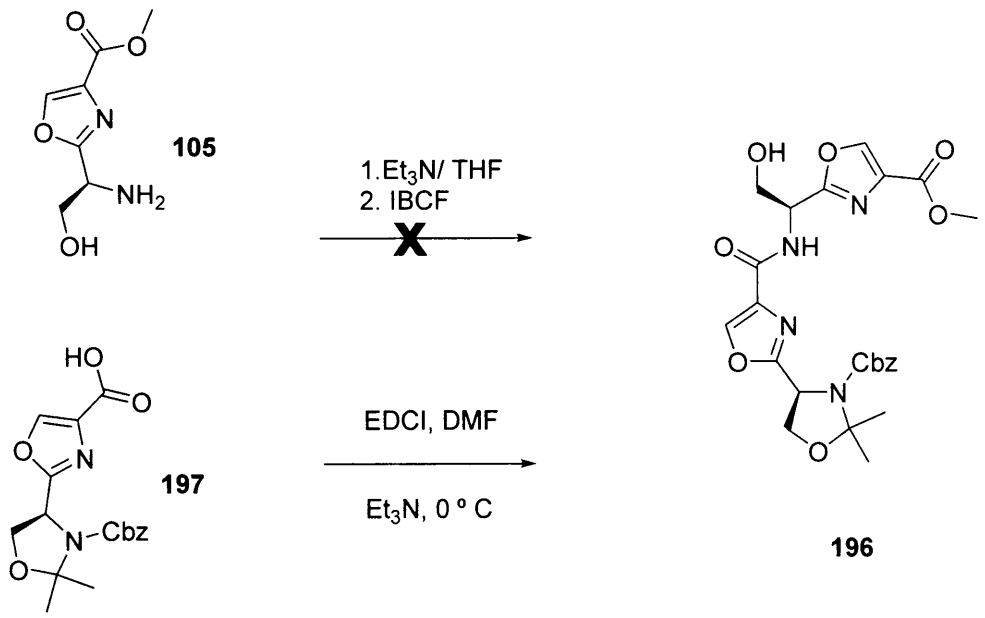
4.3.1 Acylation of the trisoxazole precursor 196

The intermediates **197** and **105** required for the synthesis of amide **196** were successfully prepared (scheme 83). Initially, a similar coupling procedure to obtain amide **200** was attempted using the intermediates (**197** and **105**), in the presence of triethylamine and isobutyl chloroformate at -30 °C. According to the tlc results, a new compound had formed and no starting material remained. The new spot on the tlc (Rf 0.7, EtOAc) was isolated; however, the NMR spectrum showed that the product formed may be an over reacted species, possibly of type **E** (figure 37).



E

Figure 37 Side-product **E**

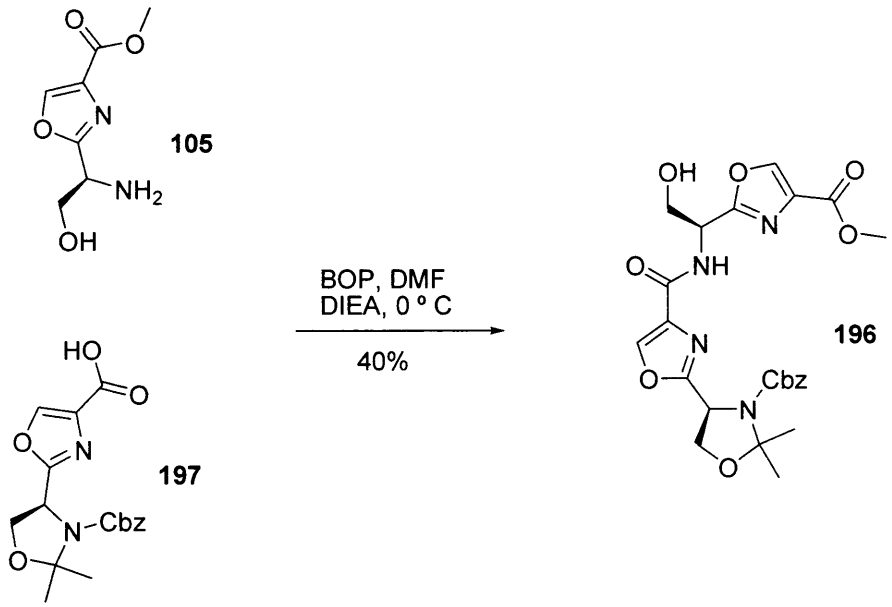


Scheme 83

The method of obtaining the hydroxy amide **196** required careful reconsideration owing to this unexpected result. Accordingly, many trial reactions were carried out, each involving 200 mg of acid **197**.

First, the temperature of the reaction was altered. The intermediates **197** and **105** were stirred at 0 ° C for 2 hours, then to room temperature with DCC. These conditions afforded two new compounds which were each isolated; the required amide **196** (Rf = 0.3 in EtOAc) in very poor yield (40%), in comparison to the side-product **E** (70%) (scheme 83). Another coupling reagent, EDCI, was used under similar conditions however, only a poor yield of 15% was obtained.

It seemed that the temperature of the reaction was not the only problem and that a new coupling agent was needed. Again, 200 mg of the acid **197** was used to try out various reagents. A more specialised coupling agent was chosen involving BOP, PyBroP and DIEA; initial results were promising since the isolated yield of **196** was increased from 15% to 40% using BOP (scheme 84).



Scheme 84

However, PyBroP only formed the side product **E.** Owing to the promising result obtained with BOP, optimisation of the conditions was attempted. The amount of BOP was varied from 1.1 to 1.5 to 2.0 equivalents; by increasing the quantity of coupling agent, a marked decrease in the yield of **196** arose (table 6). Accordingly, the number of equivalents was then kept at 1.2 (77 %, entry 5).

The solvent in the reaction involving BOP was changed from DMF to CH_2Cl_2, but the yield was no better; NMR spectra showed that only **E** was formed. Variations in temperature of the reaction and the effects are summarised below. Results showed that **E** was the thermodynamic product and the desired amide **196**[1] was the kinetically favoured product. If the temperature of the reaction was increased by just one degree above -62 ° C, the yield fell dramatically.

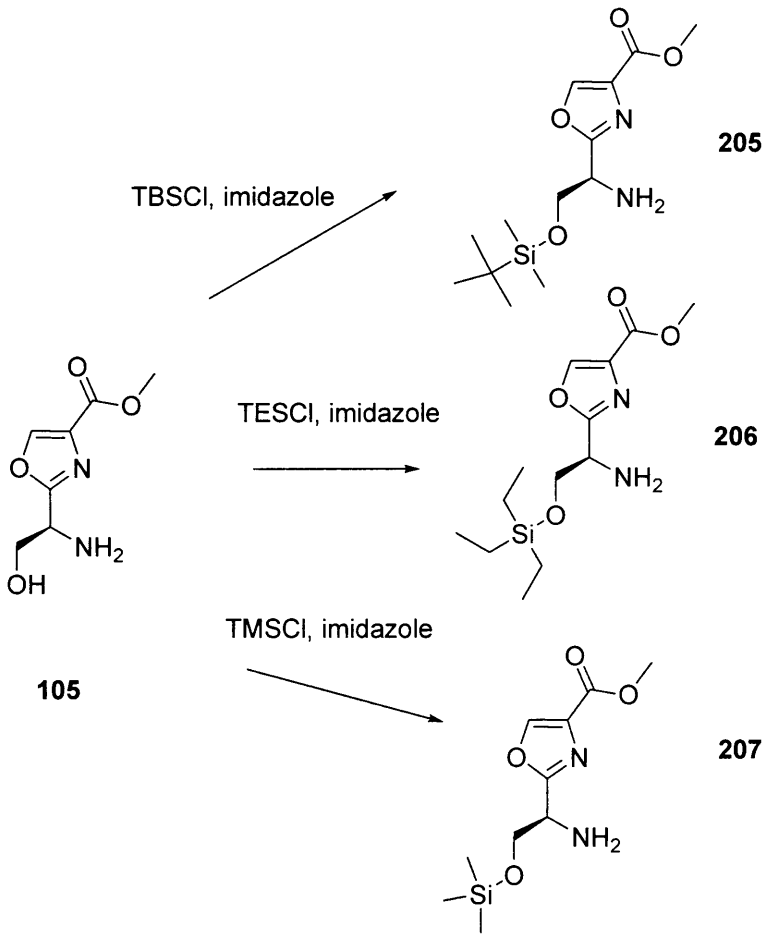
Coupling agent	Coupling agent (equivalents)	Solvent	Temperature (° C)	Time (Hours)	Yield (%)
Isobutyl chloroformate	1.1	CH_2Cl_2	-30	8	0
EDCI	1.2	CH_2Cl_2	0-rt	17	15
DCC	1.1	CH_2Cl_2	0-rt	17	40
PyBroP	1.2	DMF	-30	17	36
BOP	1.2	DMF	-30	8	36
BOP	**1.2**	**DMF**	**-62**	**72**	**77**
BOP	1.2	DMF	-15	12	40
BOP	1.2	CH_2Cl_2	-78	8	0
BOP	1.7	DMF	-62	7	40
BOP	1.1	DMF	-62	16	68
BOP	1.2	DMF	-42	16	40

Table 6. Reaction of **105** and **197** under various conditions

Endoh *et al*[1] had successfully synthesised the amide **196** using the same reagents BOP, DIEA and DMF excluding HOBT. However, the literature did not describe the conditions and so a number of trial reactions had to be carried out.

4. 3. 2 Protection of the alcohol 105 by silylation

Whilst optimising the coupling reaction, *O*-silylations were being attempted on the alcohol **105**, in the hope that if the alcohol was *O*-protected then the side product **E** would be avoided. Various silylating agents were used, including TESCl, TMSCl and TBSCl, in the presence of imidazole as the base (scheme 85). It was found that the solvent used in the protection had an effect on the yield, as summarised in table 7.

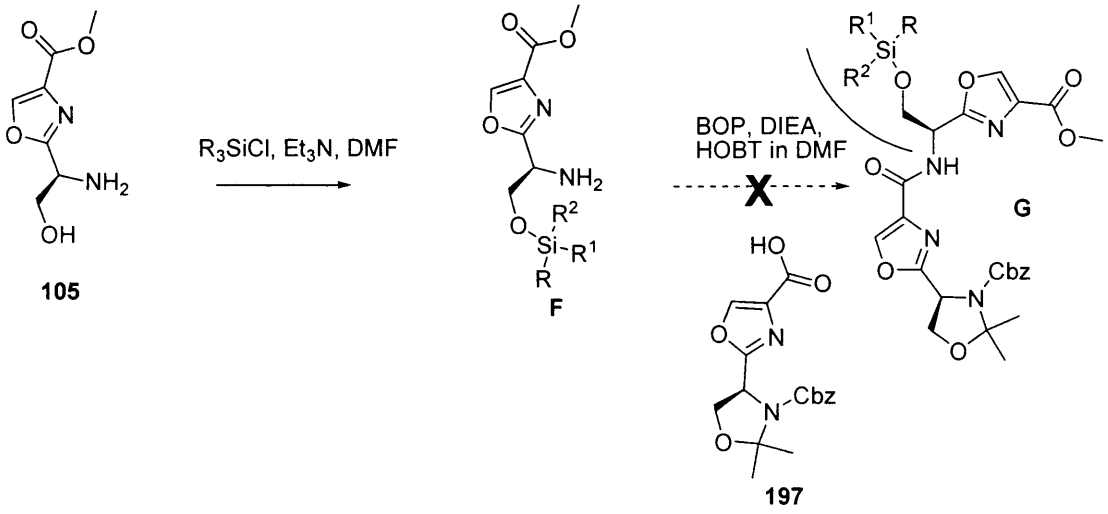


Scheme 85

	TESCl	TBSCl	TMSCl
CH_2Cl_2	50 %	46 %	60 %
DMF	19 %	62 %	35 %

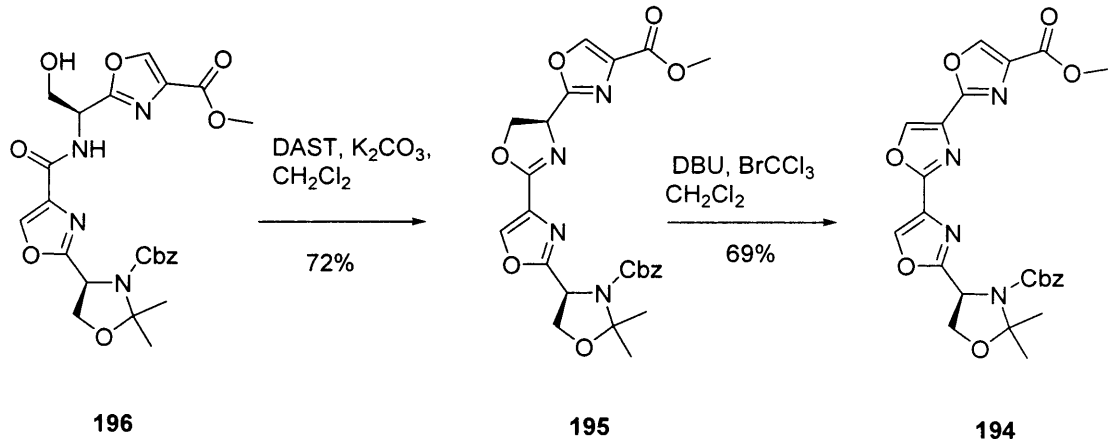
Table 7

Attempts were made to couple the protected alcohols **205**, **206**, **207** with the corresponding acid **197** using various coupling agents including; isobutyl chloroformate, DCC, EDCI and BOP. All those attempts were unsuccessful; only the coupled acid intermediates were formed, implying that the amine had failed to attack such intermediates. This may have been due to the silyl groups being quite bulky and causing steric hindrance (scheme 86).



Scheme 86

Reverting to the synthesis involving BOP (scheme 84), it was required that a larger scale acylation reaction was attempted using the amine **105** and the acid **197**. In the first trial, 1 g of **197** and 0.45 g of **105** were reacted using 1.1 equivalents of BOP. Unexpectedly, the reaction went well, giving **196** in 65% yield. The amide **196** was then reacted with DAST in CH_2Cl_2 at -78 °C to form the corresponding oxazoline **195** in 72% yield. The amidation reaction was again repeated on a 1 g scale, giving **196** (1.0 g, 83%) an improved yield.



196 **195** **194**

Scheme 87

The key tris-oxazole intermediate **194** was synthesised via the Williams-Wipf [5] procedure which included the cyclisation of the amido alcohol **196** using DAST to form **195** and then dehydrogentating it with the use of DBU and $BrCCl_3$ to form **194** with a high yield of 69% (scheme 87).

Having prepared trisoxazole **194**, different possibilities for the synthesis of **176** presented themselves, including that in (scheme 72). The common intermediate **194** of the synthesis would need to be converted into the corresponding acid **193,** and also into the amino alcohol **179** (scheme 88).

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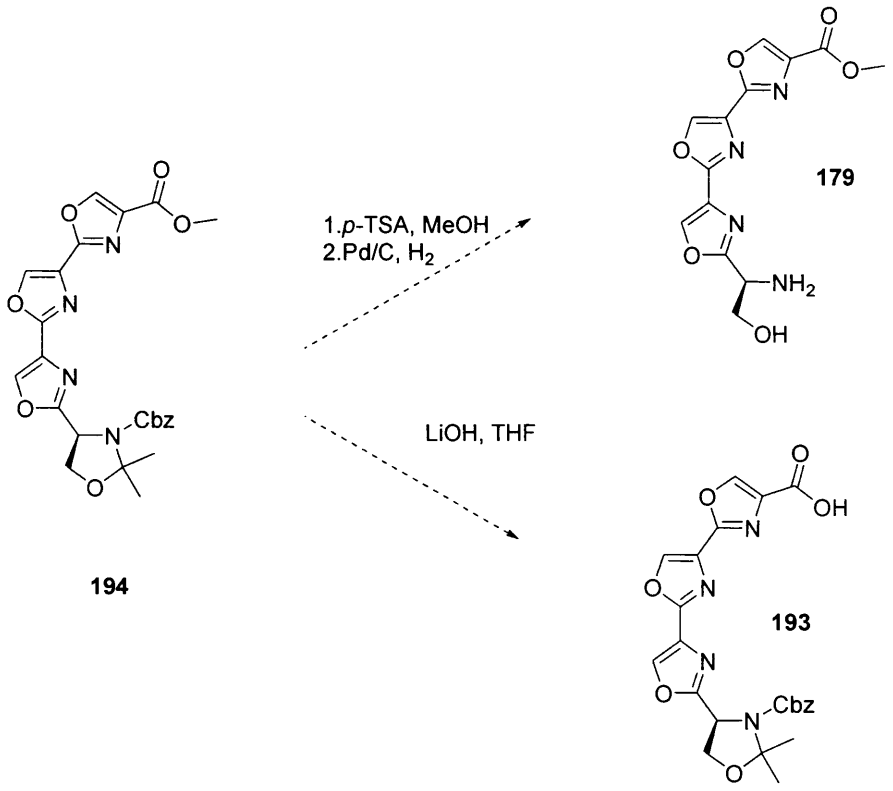


Scheme 88

Owing to the insolubility of tris-oxazoles **194** and **193**[1] another tris-oxazole derivative, which was more soluble in organic solvents was sought; the benzyl ester group **208**, was chosen to replace the methyl ester **194** (figure 38)

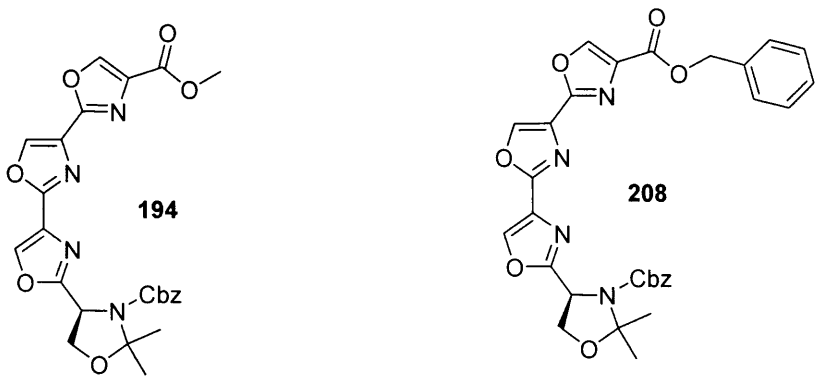
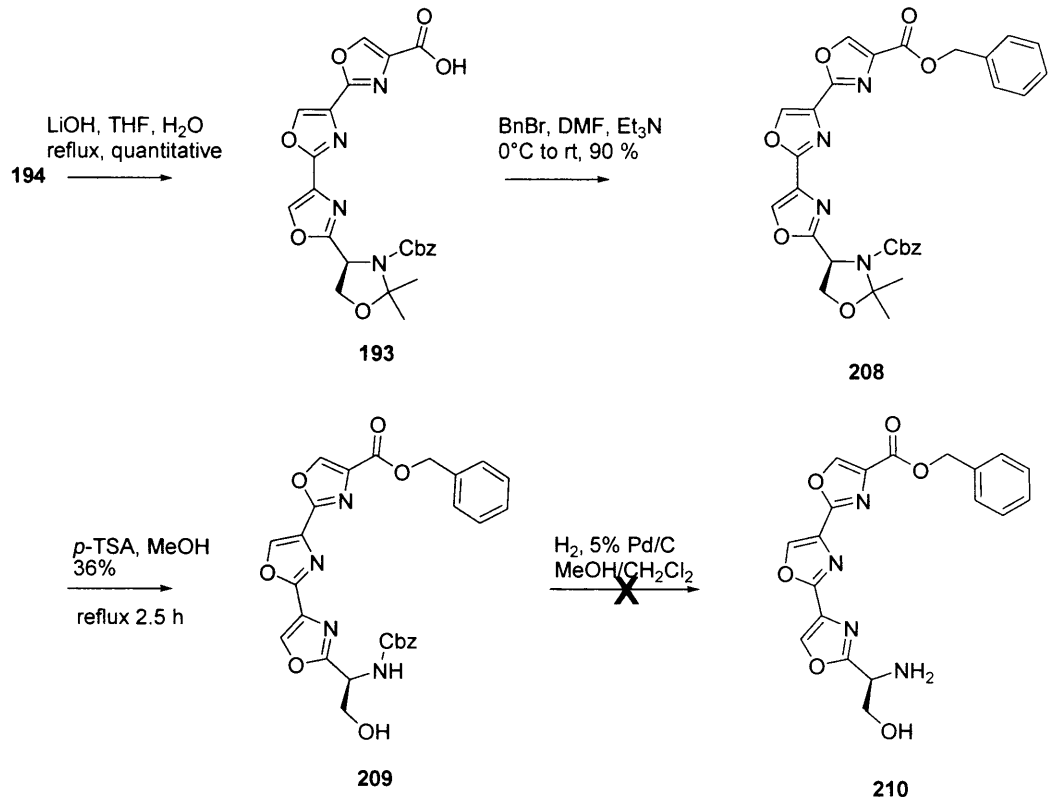


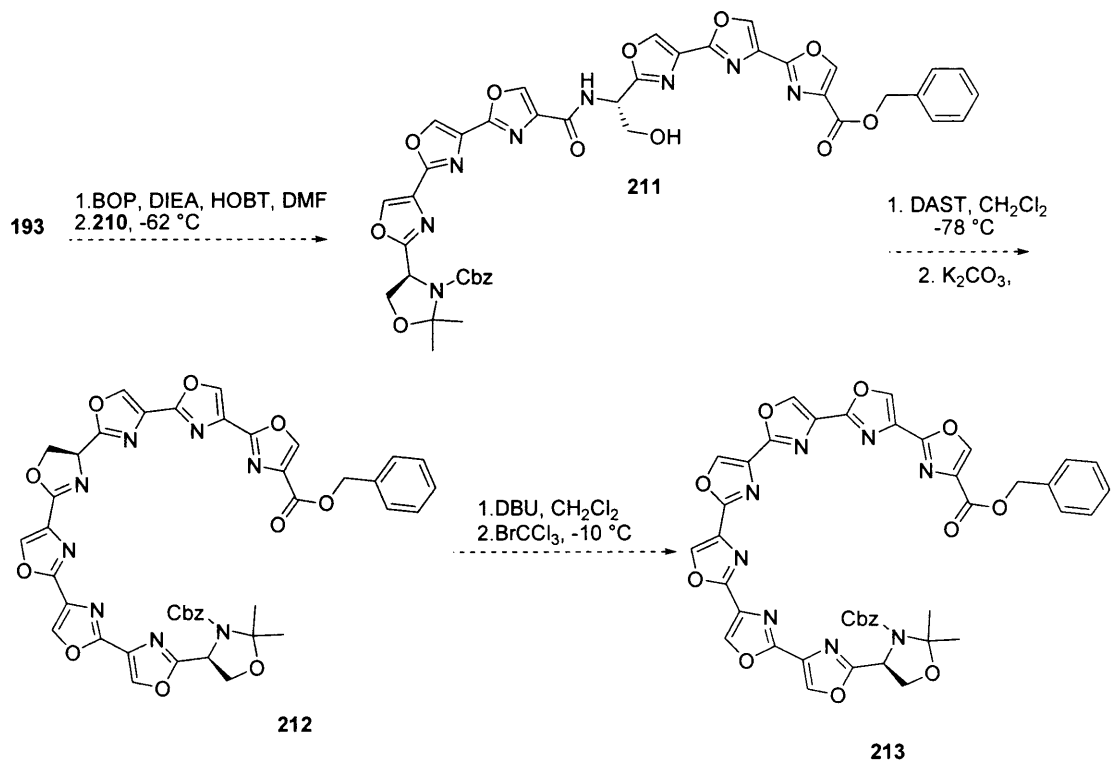
Figure 38

The benzyl ester **208** was prepared in 90% yield by reacting the acid **193** with benzyl bromide in the presence of triethylamine. The *gem*-dimethyl moiety was cleaved by *p*-TSA in methanol, but a poor yield of 36% was obtained because side-products had formed. Next, a regioselective hydrogenolysis was tried in order to remove the Cbz group of oxazolidine **209** in the presence of the benzyl ester group (scheme 89). The procedure was carried out using 5% Pd/C but took 9 hours to complete, which is quite slow owing to the use of a non-protic solvent CH_2Cl_2, although a protic solvent methanol (2:1) was also present. Tlc of the reaction showed a baseline spot, however, ^1H NMR showed no product.



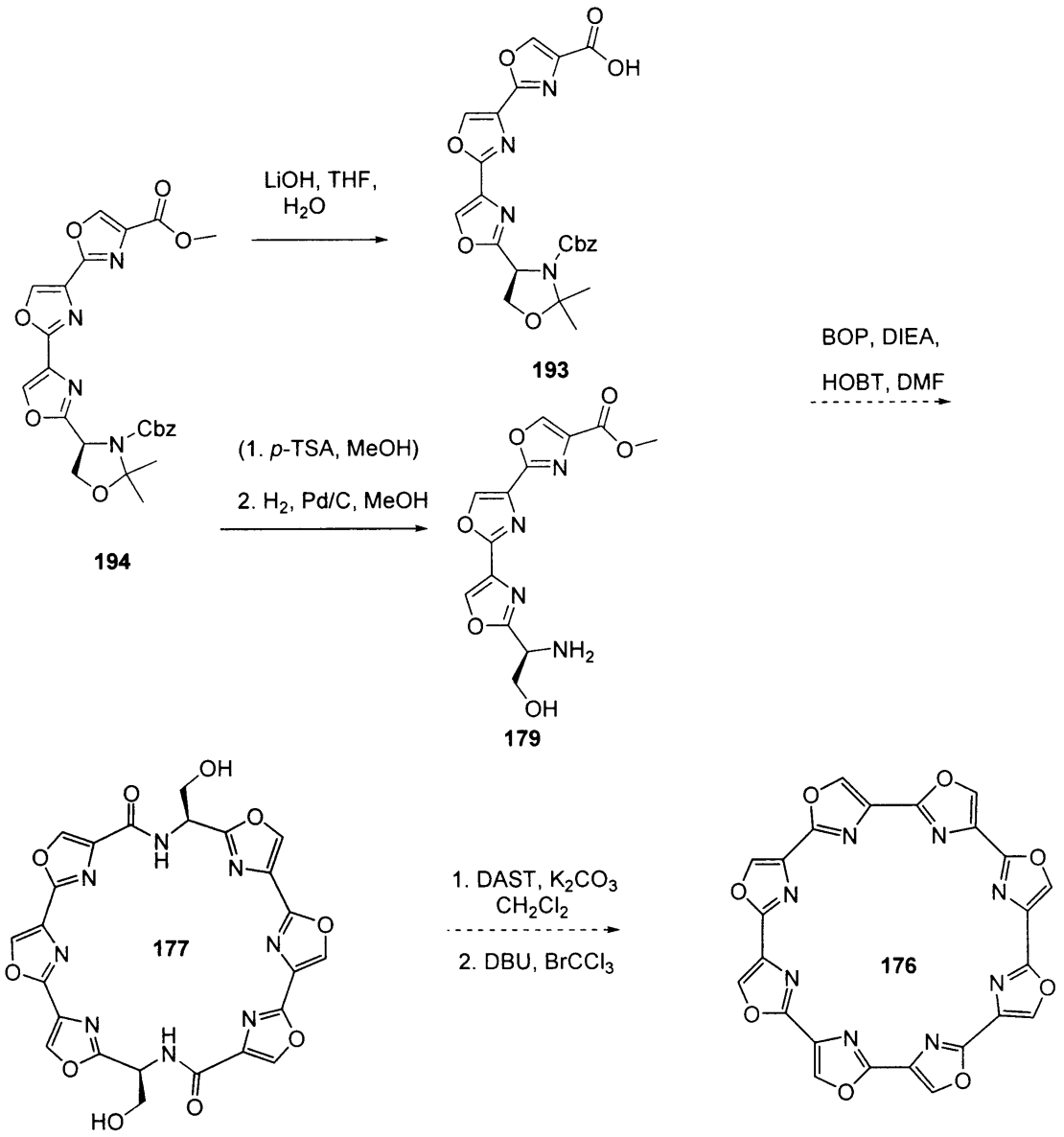
Scheme 89

Amidation of amine **210** with acid **193** was not attempted due problems in obtaining the free amine.



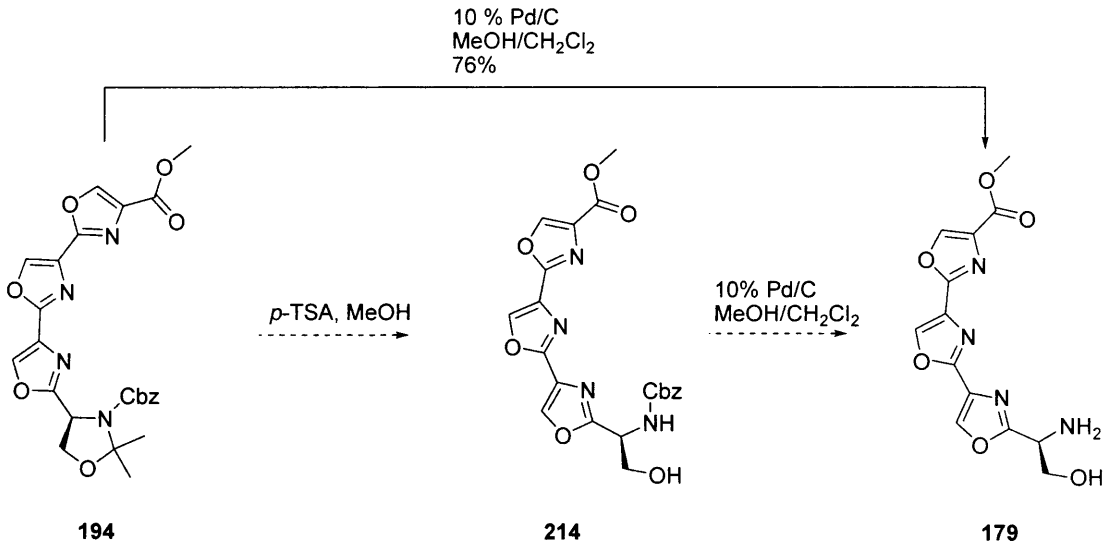
Scheme 90

Because scheme 90 had not been successful, the original sequence in scheme 91 was then pursued, in which the trisoxazole **194** needed to be deprotected to give the amino alcohol **179**.



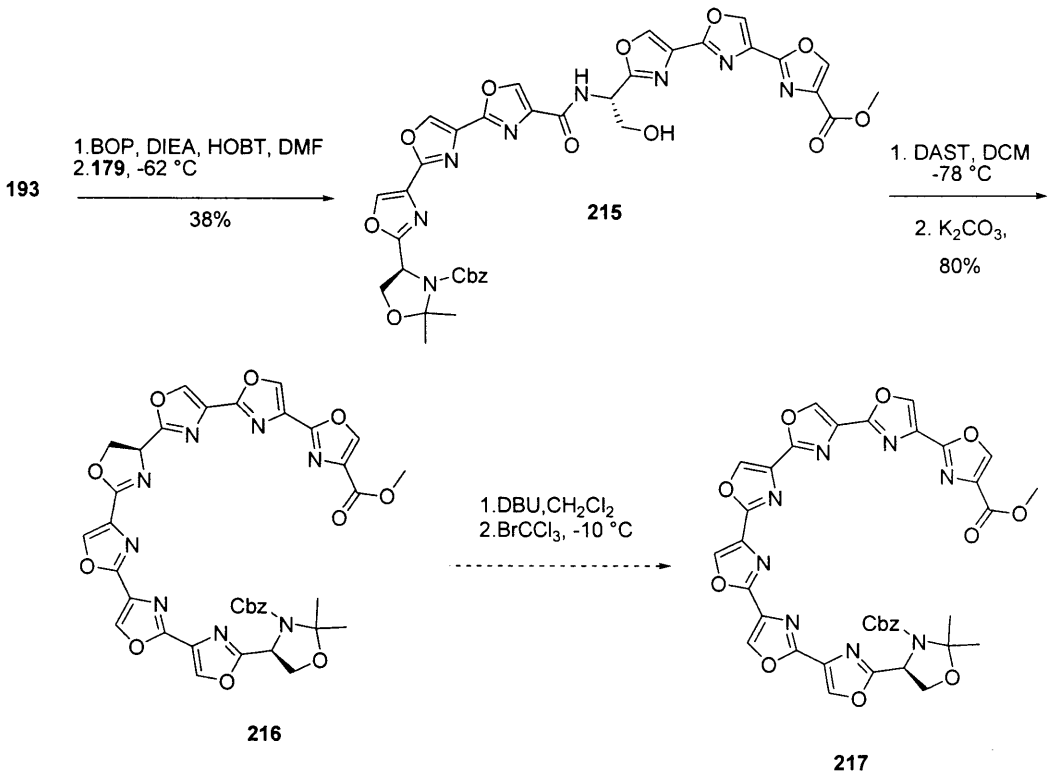
Scheme 91

Fortunately, to deprotect the *gem*-dimethyl group, hydrogenolysis over 10% Pd/C was tried first (instead of *p*-TSA) (scheme 91); tlc (EtOAc) showed the product to be baseline and the isolated product was found to be the free amino alcohol **179** obtained with no side-products. Accordingly, the synthesis of **179** was carried out in one step from **194** rather than the two shown in scheme 92. This useful result obtained in the deprotection was then applied to the earlier sequence (scheme 81) to give the amino alcohol **105**, also in high yield of 83%.



Scheme 92

The amino alcohol **179** was coupled with the acid **193** using the coupling reagents BOP, DIEA and HOBT (scheme 93). Tlc results showed only two spots had formed in the reaction, an improvement compared with using the benzyl ester **211** (scheme 90). The two spots were isolated by column chromatography, and NMR data confirmed that the product obtained with Rf =0.1 (EtOAc) was in fact the desired amide **215.**

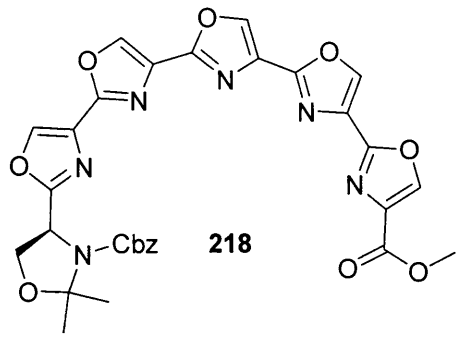


Scheme 93

The amide **215** was very insoluble in dichloromethane and hence the formation of the oxazoline **216** proved difficult. The amide **215** dissolved sparingly in dichloromethane upon heating; after cooling to –78 °C the reagents diethylamino sulfur trifluoride and potassium carbonate were added. As soon as DAST was added to the amide **215** in dichloromethane the solid dissolved and immediately the oxazoline **216** precipitated as a white solid isolated in 80% yield. The ^{1}H NMR spectrum was obtained at 120 °C in DMSO in which it was sparingly soluble. Oxazoline peaks were found at δ_H 4.85 (CH_2) and 5.28 (CH), HRMS confirmed the accurate mass. However, ^{13}C NMR data could not be obtained since the probe required for the NMR cannot be heated for long experiments; ^{13}C NMR spectra at room temperature were unresolved. Unfortunately, the synthesis of macrocycle **176** using this route had to be abandoned owing to the insolubity of oxazoline **216**.

4. 4 A third approach: synthesis of the pentaoxazole core 218

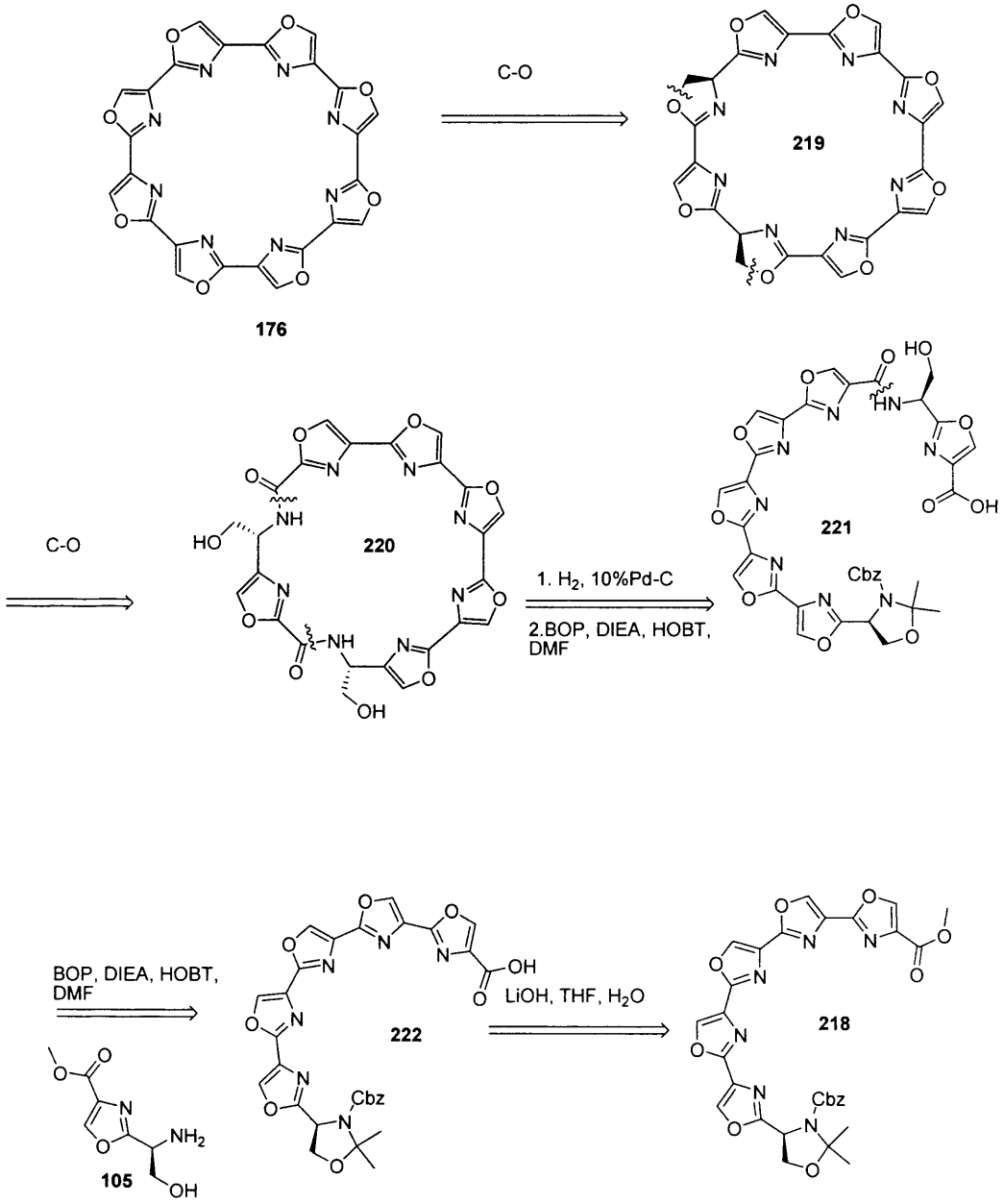
With the successful isolation of the functionalised trisoxazole fragment **194**, many different approaches to macrocycle **176** could be investigated. A third approach would require the synthesis of the penta-oxazole **218** (figure 39) which contains all the unsubstituted oxazole rings in telomestatin and five of these in macrocycle **176**. The sequence was investigated concurrently with the second approach.

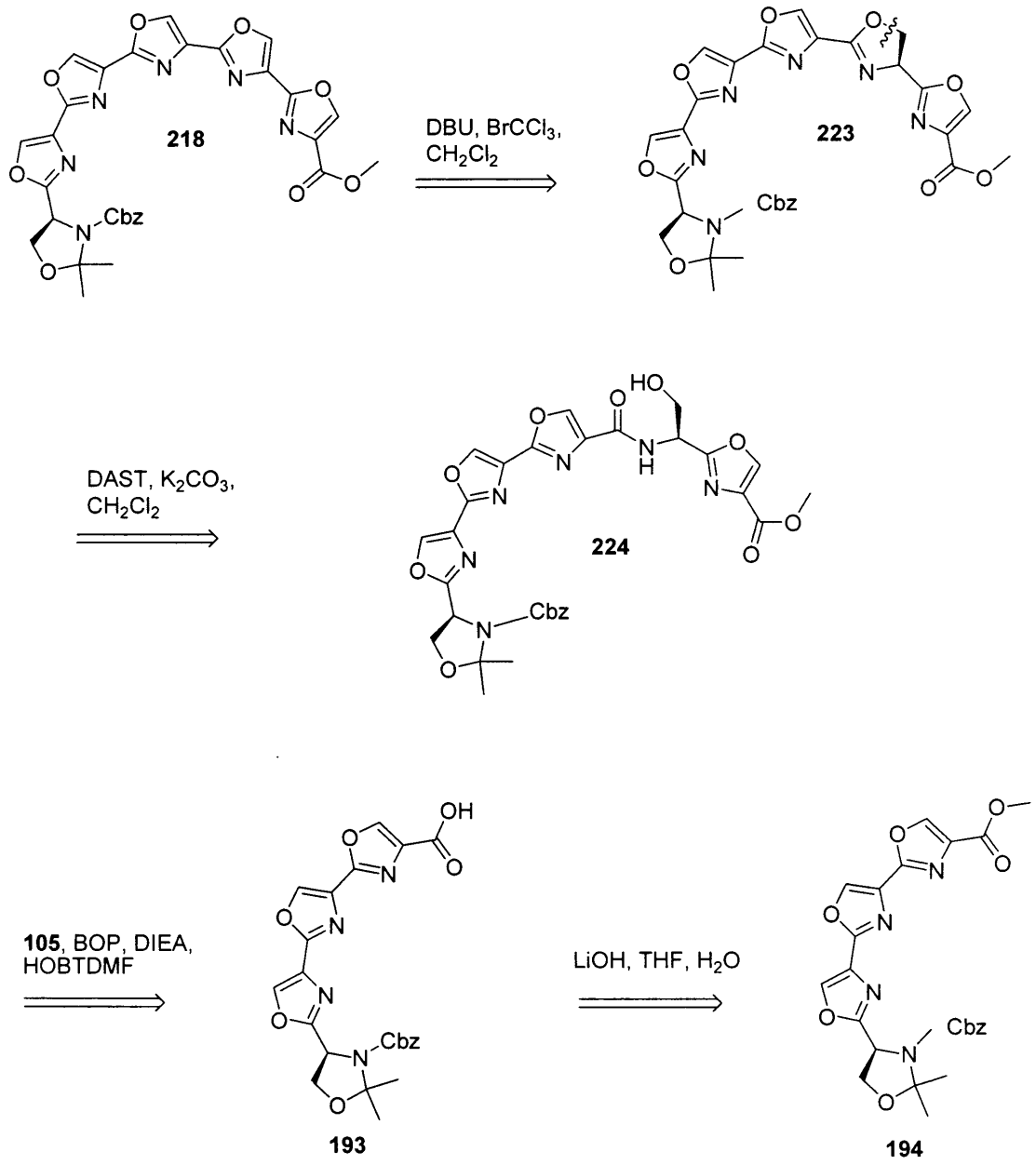


218

Figure 39

The retrosynthesis to form the penta-oxazole **218** is shown in scheme 37:

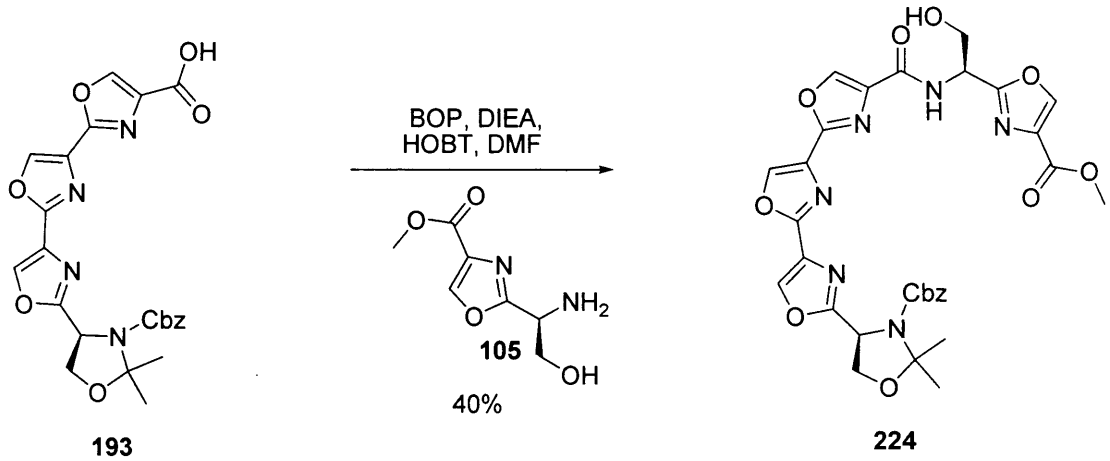




Scheme 94

The retrosynthesis to form the pentaoxazole is shown in scheme 94 and follows a similar approach to the previous two routes, in which an acid and an amino alcohol would be united to give an amide, here **224**. Then a Williams-Wipf reaction would be carried out to give the pentaoxazole **218**. Hydrolysis of ester **218** would give the acid **222**. Which would then be coupled with the amino alcohol **105**, to give the amide **221** (scheme 94) and finally a Williams-Wipf procedure would furnish macrocycle **176**.

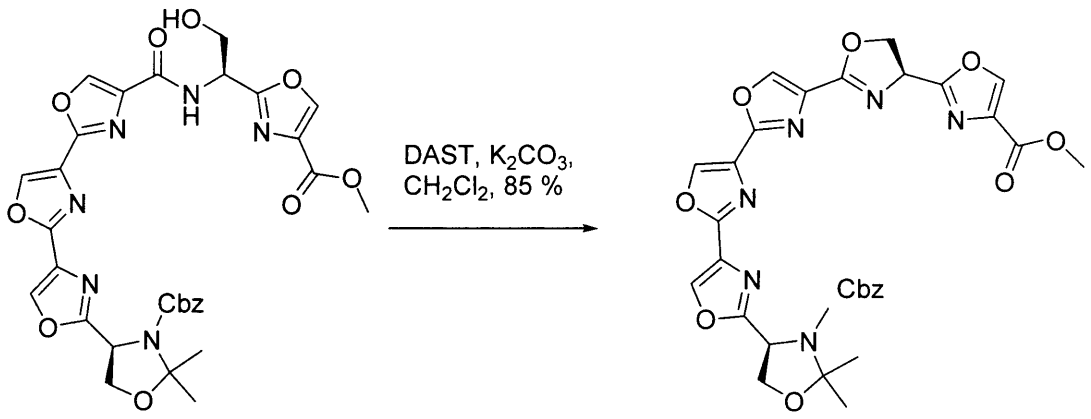
The synthesis began with the amidation of the amino alcohol **105** with the acid **193** (scheme 95). The reaction was carried out similarly to the second approach and involved BOP, DIEA, HOBT and DMF. Pattenden *et al*[10] carried out a similar procedure to synthesise the natural product YM-21613. In the synthesis a monothiazole amino alcohol was reacted with a trisoxazole acid together however, the synthesis used EDCI, NMM, HOBT in CH_2CH_2 as the reagents (Ch 1, page 41). Tlc results showed that the reaction had progressed; however, some starting material was still present even after stirring at -62 °C for 4 days. It was decided that the amide **224** would be purified via column chromatography since the starting materials in the crude material would interfere with the next step. After column chromatography, the isolated yield of **224** was 40%.



193 **224**

Scheme 95

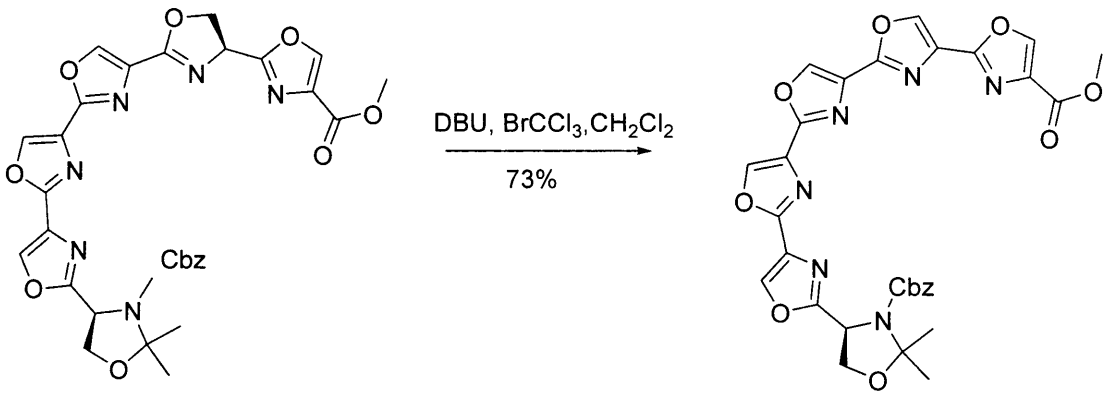
The next step involved the synthesis of the oxazoline **223**; the pure amide **224** was reacted with DAST and potassium carbonate in CH_2Cl_2 at -78 °C (scheme 97). As the reaction progressed a solid material formed during the first five minutes. After five minutes the solid foam was filtered and washed with CH_2Cl_2. ^1H NMR and HRMS data showed that oxazoline **223** had been obtained (scheme 96). However, the white foam was highly insoluble even in DMSO at 90 °C.



224 **223**

Scheme 96

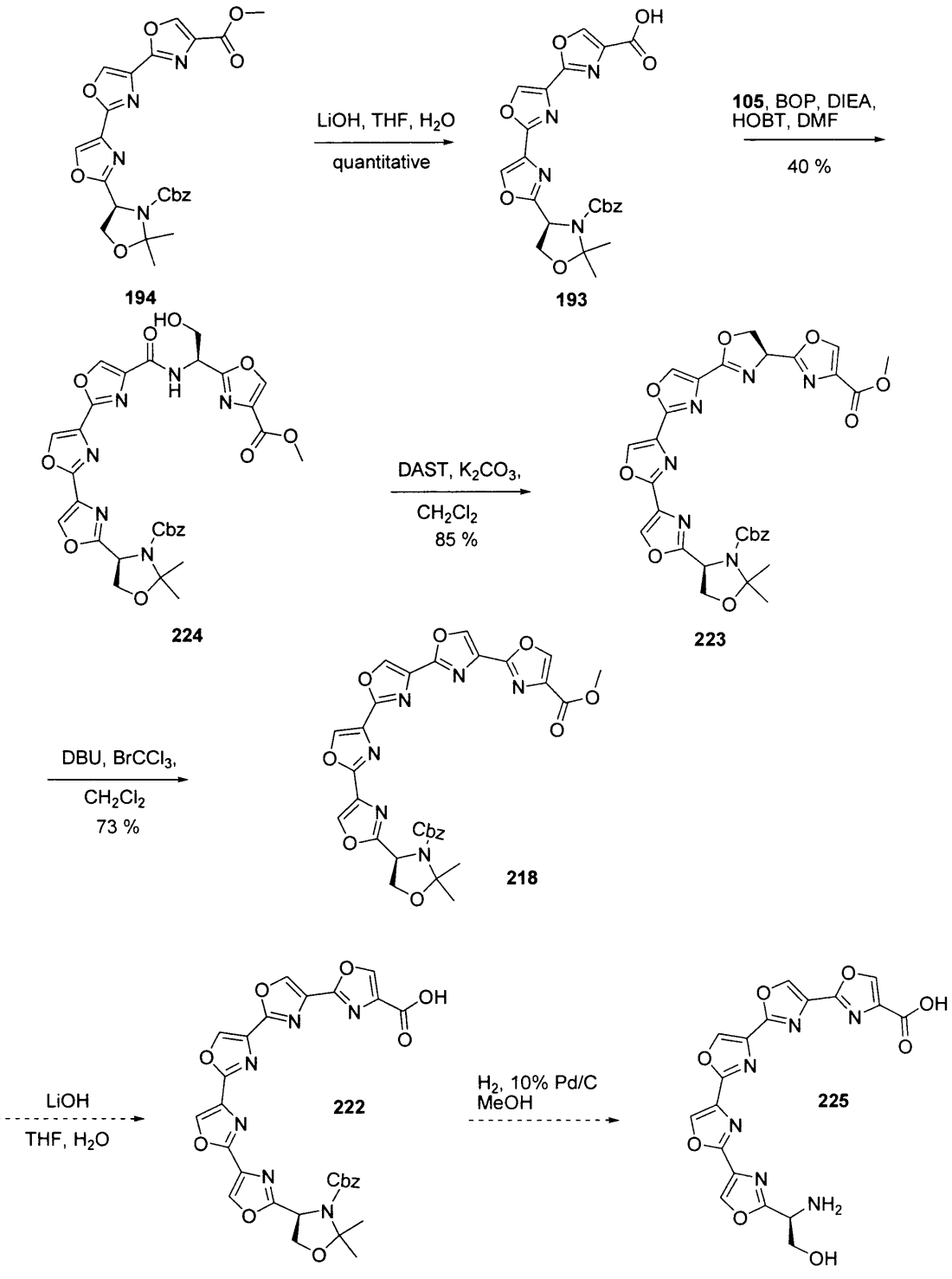
Eventually, the oxazoline **223** was dissolved in a copious amount of hot CH_2Cl_2 (30 mL). Once the sparingly soluble oxazoline **223** had been dissolved DBU and $BrCCl_3$ were added; immediately a solid material formed which was quite crystalline (scheme 97). Initially it was thought that the solid material may have been the oxazoline, but the spectral data confirmed that penta-oxazole **218**[11] had been formed. It was even more difficult to obtain a ^1H NMR spectrum for **218**, since it failed to dissolve in DMSO at 90 °C. However, at 110 °C a satisfactory ^1H NMR spectrum was obtained.

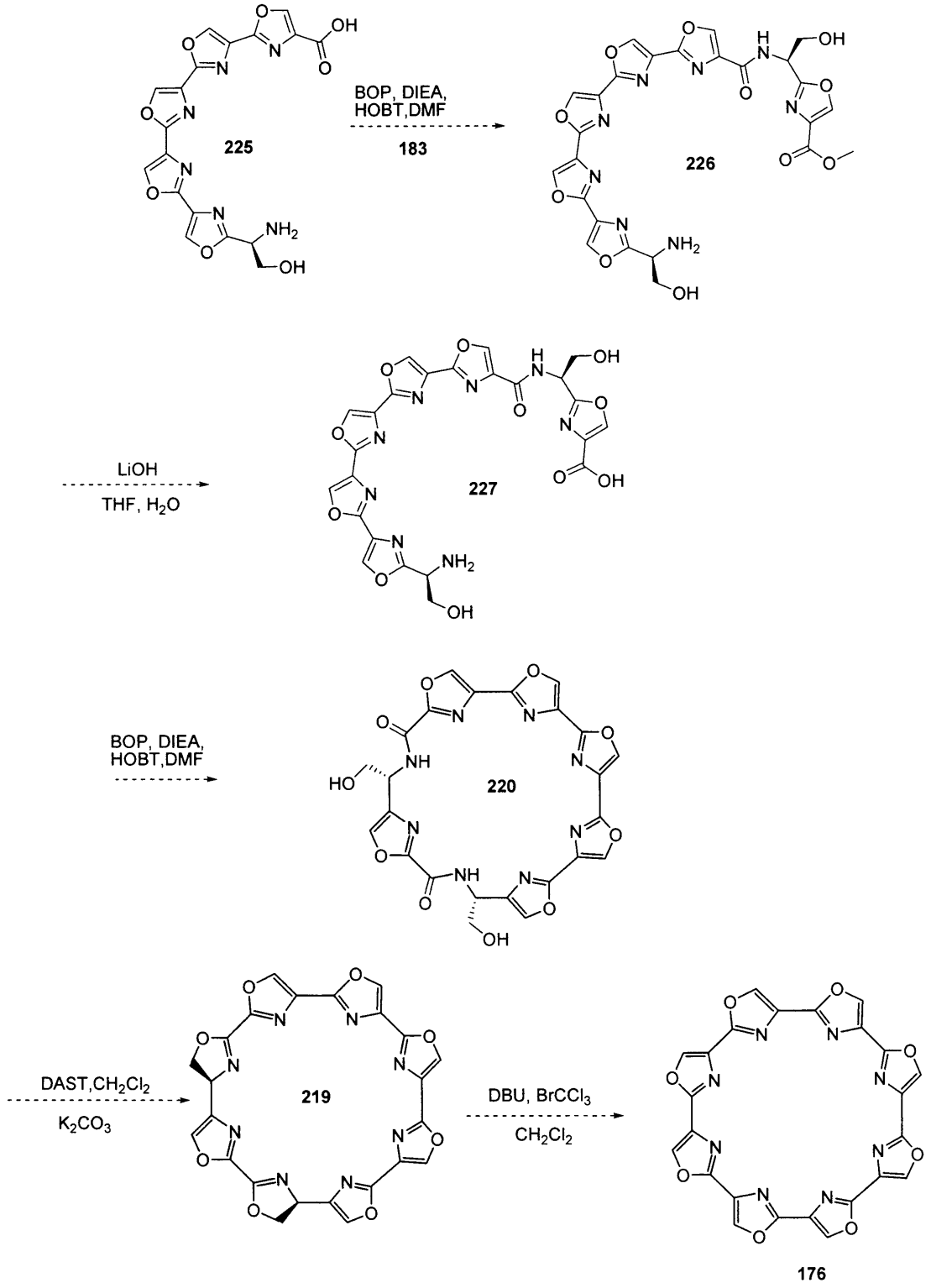


223 **218**

Scheme 97

The insolubility of **218** in DMF or DMSO made continuation of the synthesis very difficult (scheme 98). The next step would have involved the dehydrogenation of the oxazolidine to the amino alcohol **105**. However, **218** could not be dissolved in CH_2Cl_2 or methanol which led to the discontinuation of the synthesis. In summary, the synthesis went as far as the pentaoxazole **218**.

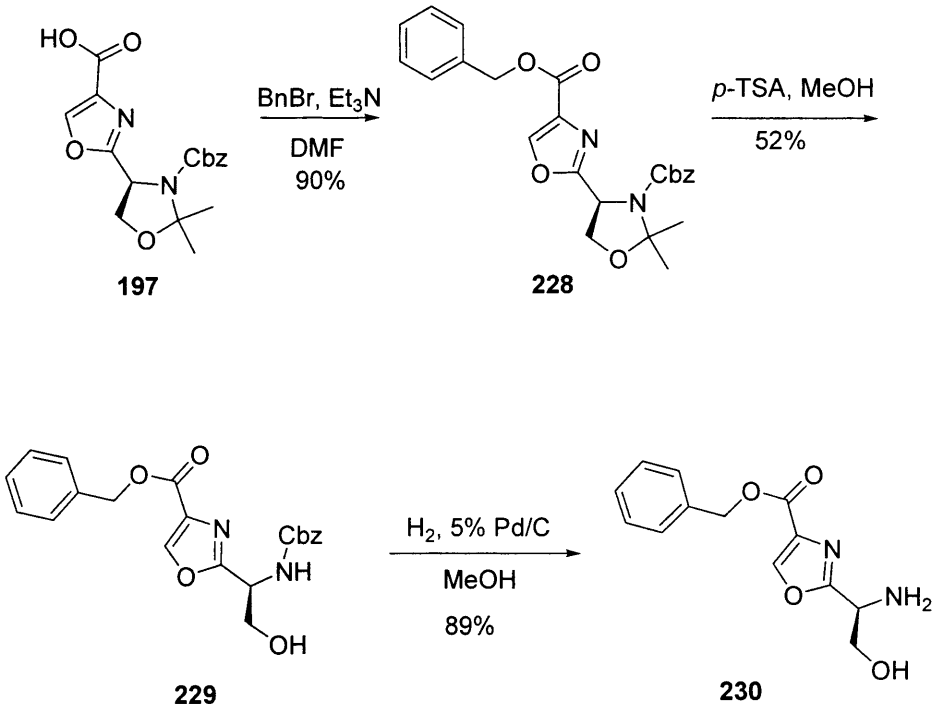

LiOH, THF, H2O
quantitative
105, BOP, DIEA, HOBT, DMF
40 %
194
193
DAST, K2CO3,
CH2Cl2
85 %
224
223
DBU, BrCCl3,
CH2Cl2
73 %
218
LiOH
THF, H2O
222
H2, 10% Pd/C
MeOH
225



Scheme 98

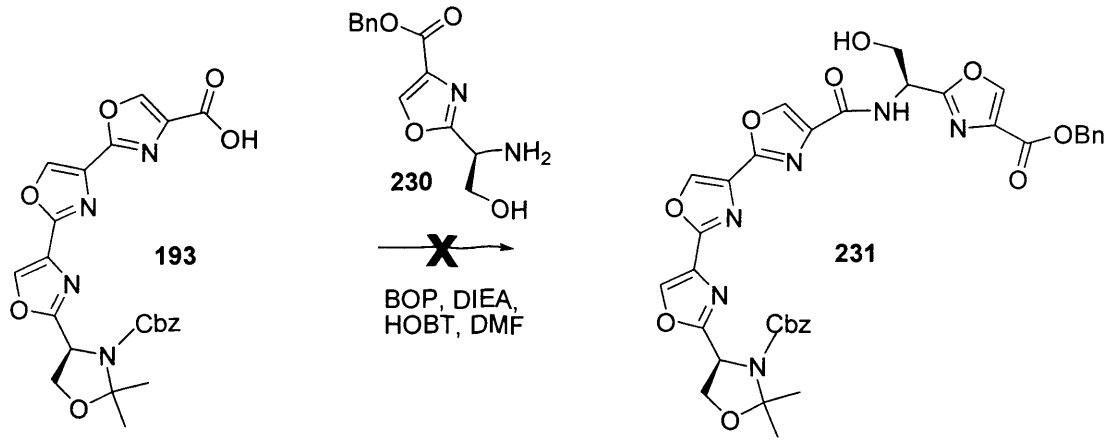
Owing to the insolubility of the pentaoxazole **218**, a more hydrophobic ester group was chosen; it was decided that a benzyl ester group should replace the methyl ester of **218,** ultimately to synthesise ester **233** (scheme 101). Manipulation of the functionality in acid **197** was satisfactorily achieved by *O*-

benzylation (benzyl bromide in the presence of triethylamine) to give ester **228**, followed by cleavage of the hemiaminal carbon atom (*p*-TSA in methanol) to give the alcohol **229**. Subsequent hydrogenolysis afforded the amino alcohol **230** (scheme 99).



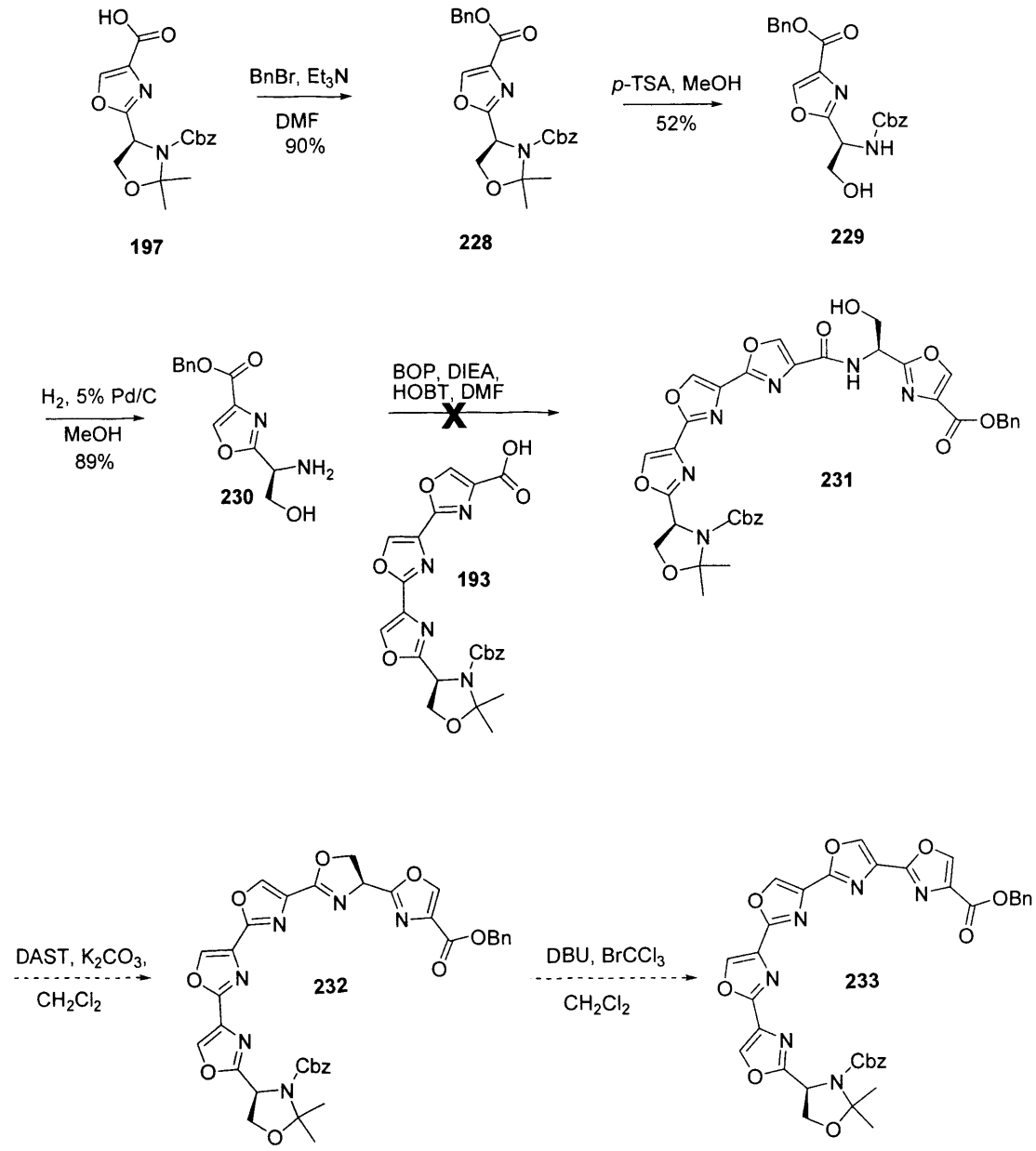
Scheme 99

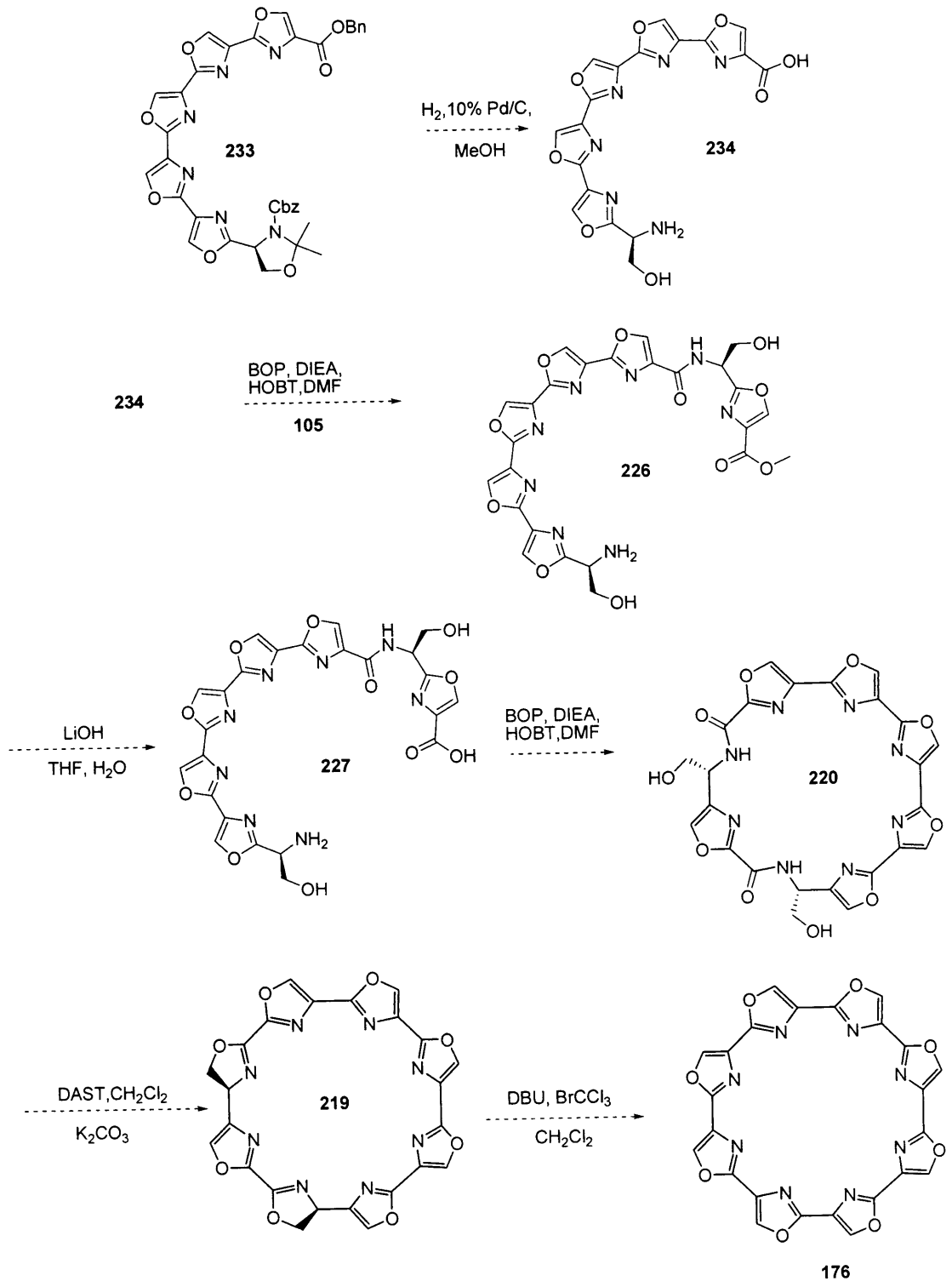
Acylation of the amino alcohol **230** with the trisoxazole carboxylic acid **193** was attempted using BOP, DIEA, HOBT in DMF (scheme 100). The temperature of the reaction was kept at – 62 ° C and stirred for 4 days. However, tlc results were quite disappointing, since there were five spots. Each compound was isolated, but ^{1}H NMR data showed none was the amide **231**. The route was discontinued owing to time constraints and lack of materials.



Scheme 100

The proposed route to macrocycle **176** using the benzyl ester amide **231** had proved very troublesome. It appeared that the acylation of amine **230** with acid **193** formed products other than the amide **231**. This failure to react indicated that the revised synthesis (scheme 101) could not be continued. Hence, routes to the macrocycle **176** via pentaoxazole intermediates were not investigated further.


HO
O
N
O
N
Cbz
O
197
BnBr, Et3N
DMF
90%
BnO
O
N
O
N
Cbz
O
228
p-TSA, MeOH
52%
BnO
O
N
O
Cbz
NH
OH
229
H2, 5% Pd/C
MeOH
89%
BnO
O
N
O
NH2
OH
230
BOP, DIEA,
HOBT, DMF
X
OH
O
N
O
N
193
N
O
Cbz
N
O
HO
O
N
H
O
N
O
N
O
N
O
OBn
O
231
N
Cbz
N
O
DAST, K2CO3,
CH2Cl2
O
N
O
N
O
N
O
N
OBn
O
232
N
Cbz
N
O
DBU, BrCCl3
CH2Cl2
O
N
O
N
O
N
O
N
OBn
O
233
N
Cbz
N
O


233
H_2,10% Pd/C,
MeOH
234
BOP, DIEA,
HOBT,DMF
105
226
LiOH
THF, H_2O
227
BOP, DIEA,
HOBT,DMF
220
DAST,CH_2Cl_2
K_2CO_3
219
DBU, $BrCCl_3$
CH_2Cl_2
176

Scheme 101

References

(1) Endoh, N.; Tsuboi, K.; Kim, R.; Yonezawa, Y.; Shin, C. *Heterocycles.* **2003**, *60*, 1567-1572.

(2) Faul, M. M.; Winneroski, L. L.; York, J. S.; Reinhard, M. R.; Hoying, R. C.; Gritton, W. H.; Dominianni, S. J. *Heterocycles.* **2001**, *55*, 689-704.

(3) Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361-2364.

(4) Chattopadhyay, S. K.; Biswas, S.; Pal, B. K. *Synthesis.* **2006**, 1289-1294.

(5) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.

(6) Doi, T.; Yoshida, M.; Shin-ya, K.; Takahashi, T. *Org. Lett.* **2006**, *8*, 4165-4167.

(7) Chhabra, S. R.; Mahajan, A.; Chan, W. C. *J. Org. Chem.* **2002**, *67*, 4017-4029.

(8) Yonezawa, Y.; Tani, N.; Shin, C. G. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1492-1499.

(9) Saito, H.; Yamada, T.; Okumura, K.; Yonezawa, Y.; Shin, C. G. *Chem. Lett.* **2002**, 1098-1099.

(10) Deeley, J.; Pattenden, G. *Chem. Commun.* **2005**, 797-799.

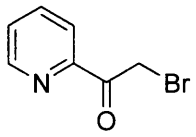
(11) Marson, C. M.; Saadi, M. *Org. Biomol. Chem.* **2006**, *4*, 3892-3893.

Chapter 5

EXPERIMENTAL

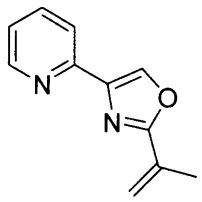
General: Melting points were determined on a microscope hot-stage Electrothermal 9100 apparatus, and are uncorrected. Infra-red (IR) spectra were recorded on a Perkin-Elmer PE-983 spectrophotometer; absorptions are quoted in wavenumbers (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded on the Brucker AC300 (300MHz) spectrometer; data reported in parts per million (δ), with tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) are given in Hertz (Hz). The following abbreviations are used in signal assignments: singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m). ^{13}C NMR spectra were obtained using a Brucker AMX 300 (75MHz), Brucker AMX 400 (100 MHz), Brucker AMX 500 (125 MHz), spectrometer and are recorded in parts per million (δ) with $CHCl_3$ as an internal standard. Mass spectra were recorded on a VG7070H mass spectrometer with Finigan Incos II data system at University College London. Thin-layer chromatography was performed on Merck 0.2mm aluminium-backed silica gel 60 F_{254} plates and visualised by ultra violet light or an alkaline potassium permanganate spray with subsequent heating. Flash chromatography was performed using Merck 0.040-0.063 mm, 230-400 mesh silica gel. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Evaporation refers to the removal of the solvent under reduced pressure. Glassware, syringes and needles for moisture-sensitive reactions were pre-dried in an oven (130°C). Temperatures below 0 °C were obtained from various mixtures of water, freezing salt and ice, acetone and ice, acetone and dry ice, or liquid nitrogen. Measurements of optical rotation were obtained on AA Series Automatic polarimeter (PolAAR 2000) at 23 °C.

2-Bromo-1-pyridin-2-yl ethanone (81)[1]



To a solution of 2-acetylpyridine (2.00 g, 16.5 mmol) in benzene (20 mL), was added glacial acetic acid (5 mL, 87 mmol). Bromine (0.84 mL, 16.8 mmol: **CAUTION**) in benzene (12.5 mL) was added to the reaction mixture over 10 min. A yellow precipitate formed; after stirring for 48 h the mixture was filtered and dissolved in saturated potassium carbonate (20 mL). The orange oil formed was dissolved in diethyl ether (20 mL) and extracted. The organic layer was separated, dried over $MgSO_4$, filtered and evaporated to give **81** (2.80 g, 89%) as a brown oil; Rf 0.5 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.68 (1H, ddd, *J*= 0.88, 1.60, 4.74 Hz, pyridyl), 8.07 (1H, ddd, *J*= 0.92, 2.10, 7.8 Hz, pyridyl), 7.88 (1H, ddd, *J*= 1.72, 7.6, 9.3 Hz, pyridyl), 7.52 (1H, ddd, *J*= 1.25, 4.76, 7.6 Hz, pyridyl), 4.84 (2H, s, C*H*$_2$Br); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 192.49 (*C*=O), 151.46 (s, pyridyl), 149.16 (d, pyridyl), 137.15 (d, pyridyl), 127.78 (d, pyridyl), 122.69 (d, pyridyl), 32.23 (*C*H$_2$Br).

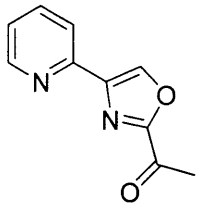
2-(1- Isopropenyl)-4-(2-pyridyl)oxazole (52)[1]



2-Bromo-1-pyridin-2-yl ethanone (**81**) (0.42 g, 2.10 mmol) and methacrylamide (0.35 g, 4.11 mmol) were dissolved in THF (15 mL) in a reinforced tube. The tube was sealed using a polythene cap and heated at 100 ℃ using a paraffin oil bath for 72 h. After being cooled to room temperature, the tube was opened (**CAUTION**) and the mixture was evaporated. The residue was purified by column chromatography (dichloromethane) to give **52** (0.29 g, 75 %) as a brown

oil; Rf 0.3 EtOAc; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.57(1H, ddd, *J*= 1.0, 1.7, 4.8 Hz, pyridyl), 8.19 (1H, s, CH, oxazolyl), 7.92 (1H, ddd, *J*= 0.92, 1.9, 8.0 Hz, pyridyl), 7.73 (1H, ddd, *J*= 1.0, 7.6, 9.3 Hz, pyridyl), 7.19 (1H, ddd, *J*= 1.0, 4.8, 7.5 Hz, pyridyl), 6.01 (1H, s, C=C*H*), 5.41 (1H, s, C=C*H*), 2.20 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 162.93 (s, pyridyl), 150.85 (O-*C*=N, oxazolyl), 149.38 (d, pyridyl), 136.84 (C-*C*=C), 136.47 (C=*C*-O, oxazolyl), 131.69 (C=*C*-N, oxazolyl), 122.68 (d, pyridyl), 120.33 (d, pyridyl), 119.13 (d, pyridyl), 118.47 ($C=CH_2$), 18.98 (CH_3).

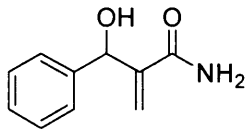
2-Acetyl-4-(2-pyridyl)oxazole (79)[1]



Method A: To a solution of 2-(1-methylethenyl)-4-(2-pyridyl) oxazole (**52**) (0.33 g, 1.77 mmol) in dioxane (75 mL) and water (75 mL) was added 5 drops of an OsO_4 solution in water and sodium periodate (1.30 g, 6.1 mmol). The reaction mixture was left to stir at 50 ℃ over 16 h. Dichloromethane (30 mL) was added to the reaction mixture and washed with aqueous sodium metabisulfite (30 mL), water (30 mL) and brine (20 mL), the organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (dichloromethane) to afford **79** (0.14 g, 42 %) as a white solid; mp 121-123 °C, lit.[1] mp 122-123.5 °C ; Rf 0.25 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.61 (1H, ddd, *J*= 0.9, 1.76, 4.8 Hz, pyridyl), 8.42 (1H, s, oxazolyl), 7.97 (1H, ddd, *J*= 0.98, 2.0, 7.9 Hz, pyridyl), 7.79 (1H, ddd, *J*= 1.78, 7.6, 9.4 Hz, pyridyl), 7.29 (1H, ddd, *J*= 1.2, 4.8, 7.5 Hz, pyridyl), 2.74 (3H, s, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 185.65 (CH_3C=O), 157.43 (s, oxazolyl), 149.58 (s, pyridyl), 142.67 (d, pyridyl), 139.66 (d, oxazolyl), 137.09 (s, oxazolyl), 127.67 (d, pyridyl), 123.52 (d, pyridyl), 120.51 (d, pyridyl), 26.7 (CH_3).

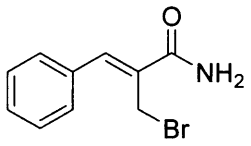
Method B: To a solution of 2-(1-methylethenyl)-4-(2-pyridyl) oxazole (**52**) (2.18 g, 11.7 mmol) in dichloromethane (150 mL). A bubbler was placed inside the solution and ozone gas was added to the solution at -78 ℃ for 1 h. Then the reaction was purged with oxygen for 10 min. Triphenylphosphine (9.20 g, 35.1 mmol) was added to the reaction until the reaction mixture turned yellow. The reaction was left to stir for 16 h at ambient temperature. The residue was purified by column chromatography (dichloromethane) to afford **79** (1.32 g, 60%) as a white solid

2-(Hydroxy- phenyl-methyl)acrylamide (85)[2]



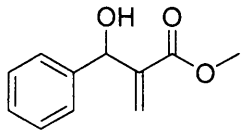
To a solution of benzaldehyde (4.79 mL, 49.0 mmol) in methanol (4 mL) was added acrylamide (3.34 g, 47.0 mmol) and 3-hydroxyquinuclidine (2.98 g, 23.5 mmol). The mixture was stirred at 20 °C for 72 h. The mixture was evaporated and the residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give **85** (2.18 g, 40 %) as a white solid, mp 98-100 °C, lit.[2] mp 97-99 °C; Rf 0.2 (1:5 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, CD_3OD-d_4) δ_H 7.29 (5H, m, aryl), 6.25 (1H, s, C=C*H*), 5.66 (1H, s, C=C*H*), 5.63 (1H, s, C*H*OH); ^{13}C NMR (75 MHz, CD_3OD-d_4) δ_c 172.42 (NH_2-*C*=O), 147.66 (*C*=CH_2), 143.08 (s, aryl), 129.50 (d, aryl), 128.81 (d, aryl), 127.61 (d, aryl), 121.06 (C=*C*H_2) 74.16 (*C*HOH).

2-Bromomethyl-3-phenylacrylamide (86)



2-(Hydroxy-phenylmethyl)-acrylamide (**85**) (0.50 g, 2.8 mmol) was dissolved in hydrobromic acid (3.08 mL, 57.0 mmol). The mixture was stirred at 20 °C over 16 h. The mixture evaporated and the residue was purified by column chromatography (ethyl acetate) to give **86** (0.56 g, 83%) as a brown oil; IR (thin film) (ν_{max}) 3050, 2983, 1650, 1468, 1356, 630 cm^{-1}; Rf 0.4 (1:3 EtOAc: petroleum ether); ^1H NMR (300 MHz, $CDCl_3$) δ_H 7.77 (1H, C=C*H*), 7.46 (5H, m, aryl), 4.43 (2H, s, CH_2Br); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 167.96 (HN-*C*=O), 136.38 (*C*=CH), 134.57 (s, aryl), 132.55 (H*C*=CCH_2Br), 129.04 (d, aryl), 128.83 (d, aryl), 128.34 (d, aryl), 28.83 (*C*H_2Br); LRMS m/z (EI) 241 (M^{81}+H, 11%), 239 (M^{79}+H, 12%), 160 (100%), 143 (23%), 132 (7%), 115 (97%), 91 (13%), 80 (9%): HRMS calcd for $C_{10}H_{10}BrNO$ 238.9946. Found 238.9949.

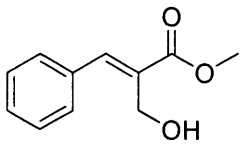
2-Methyl(hydroxyphenylmethyl)-acrylate 87[3]



To a solution of benzaldehyde (4.79 mL, 47.0 mmol) in chloroform (3 mL) was added 3-hydroxyquinuclidinine (0.29 g, 2.3 mmol). The mixture was stirred at 20 °C for 5 min. Methyl acrylate (6.39 mL, 71.0 mmol) was then added and the mixture was stirred at 20 °C over 72 h. The mixture was evaporated and the residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give **87** (8.58 g, 95 %) as a colourless oil; Rf 0.32 (1:5 EtOAc: petroleum ether); ^1H NMR (300 MHz, $CDCl_3$) δ_H 7.28 (5H, m, aryl), 6.32 (1H, s, C=C*H*), 5.84 (1H, s, C=C*H*), 5.54 (1H, s, O*H*), 3.69 (3H, s, OCH_3); ^{13}C NMR

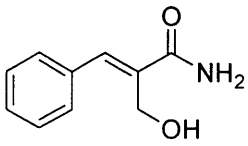
(75 MHz, $CDCl_3$) δ_c 166.75 (C=O), 142.04 (s, aryl), 141.34 (s, *C*=CH_2), 128.42 (d, aryl), 127.81 (d, aryl), 126.62 (d, aryl), 126.62 (d, C=*C*H_2), 73.14 (*C*HOH), 51.94 (O*C*H_3).

3-Hydroxymethyl-3-phenylacrylate (88)[4]



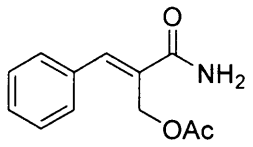
To a stirred solution of methyl 2-(hydroxyphenylmethyl)acrylate **87** (1.0 g, 5.2 mmol) and acetic anhydride (0.60 mL, 6.2 mmol) in dichloromethane (20 mL) was added trimethylsilyl trifluoromethanesulfonate (0.1 mL) at 20 °C. After 2 h, the mixture was evaporated and methanol (20 mL), potassium carbonate (2.16 g) were added. The mixture was stirred for 1h at 20 °C. Then mixture was evaporated, diluted with water (50 mL) and extracted with diethyl ether (50 mL). The organic layer was dried over $MgSO_4$, filtered and evapoarated. The residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give **88** (0.77 g, 77 %) as a colourless oil; Rf 0.5 (1:2 EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 7.83 (1H, s, C=C*H*), 7.42 (5H, m, aryl), 4.40 (2H, s, CH_2OH), 3.87 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 168.46 (C=O), 142.68 (*C*=CH), 134.52 (s, aryl), 130.92 (C=*C*H), 129.61 (d, aryl), 129.27 (d, aryl), 128.61 (d, aryl), 57.90 (*C*H_2OH), 52.21 (OCH_3).

2- Hydroxymethyl-3-phenyl-acrylamide (89)



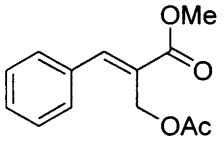
To 3-hydroxy methyl-3-phenyl-acrylate **88** (0.2 g, 1.0 mmol) was added 0.88 aqueous ammonia (3 mL). The mixture was stirred at 50 °C for 16 h. The mixture was evaporated, the residue was purified by column chromatography (ethyl acetate) to give **89** (0.14 g, 76 %) as colourless oil. IR (thin layer) (ν_{max}) 3400, 2923, 1633, 1593, 1402, 989 cm^{-1}; Rf baseline (EtOAc); ^1H NMR ($CDCl_3$, 300 MHz) δ_H 7.48 (1H, s, C=C*H*), 7.33 (5H, m, aryl), 7.28 (2H, m, NH_2), 4.28 (2H, s, CH_2OH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_c 170.30 (H_2N-C=O), 136.30 (*C*=CH), 135.93 (s, aryl), 135.76 (C=*C*H), 129.32 (d, aryl), 129.22 (d, aryl), 128.29 (d, aryl), 57.20 (*C*H_2OH); LRMS m/z (EI) 177 (M^+, 14%), 160 (23%), 148 (21%), 131 (100%), 115 (51%), 103 (42%), 91 (11%): HRMS calcd for $C_{10}H_{11}NO_2$ 177.0784. Found 177.0787.

Acetic acid 2-carbamoyl-3-phenyl-allyl ester (90)



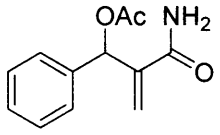
To a stirred solution of 2-(hydroxyphenylmethyl)-acrylamide (**85**) (0.50 g, 2.8 mmol) and acetic anhydride (0.33 mL, 3.38 mmol) in dichloromethane (1 mL) was added trimethylsilyl trifluoromethanesulfonate (0.1 mL) at 20 °C. After 24 h, the mixture was evaporated to leave a yellow residue which was purified by column chromatography (1:2.5 ethyl acetate: petroleum ether) to give **90** (0.55 g, 89%) as a yellow oil: IR (thin film) (ν_{max}) 3384, 1664, 1595, 1450, 1031 cm^{-1}; Rf 0.1 (EtOAc); ^1H NMR (CD_3OD, 300 MHz) δ_H 7.66 (1H, s, C=C*H*), 7.40 (5H, m, aromatic-H), 4.90 (2H, s, CH_2OAc), 2.10 (3H, s, OCH_3); ^{13}C NMR (75 MHz, CD_3OD) δ_c 172.66 (H-N-*C*=O), 172.58 (O-*C*=O), 142.15 (*C*=CH), 141.49 (C=*C*H), 135.98 (s, aryl), 131.74 (d, aryl), 130.31 (d, aryl), 129.77 (d, aryl), 60.80 (*C*H_2OAc), 20.83 (O*C*H_3); LRMS m/z (EI) 219 (M^+, 24%), 159 (100%), 148 (14%), 131 (89%), 115 (96%), 103 (53%), 91 (18%); HRMS calcd for $C_{12}H_{13}NO_3$ 219.0895. Found 219.0891.

Methyl 2-acetoxymethyl-3-phenyl-3-phenylacrylate (91)[5]



To a solution of methyl (2-hydroxyphenylmethyl)-acrylate (**87**) (0.50g, 2.8 mmol) in dichloromethane (20 mL) was added acetic anhydride (0.33 mL, 3.4 mmol) and trimethylsilyl trifluoromethanesulfonate (0.056 mL). The mixture was stirred for 2 h at 20 °C. The mixture was evaporated and the residue was purified by column chromatography (1:5 ethyl acetate: petroleum ether) to give **91** (1.10 g, 96 %) as a colourless oil; Rf 0.2 (1:5 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.97 (1H, s, C=C*H*), 7.36 (5H, m, aryl), 4.94 (2H, s, CH_2OAc), 3.82 (3H, s, OCH_3), 2.07 (3H, s, OC=OCH_3, acetate); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 170.74 (C=O, acetate), 167.30 (C=O, methyl ester), 145.50 (*C*=CH), 134.19 (s, aryl), 129.60 (C=*C*HCH_2), 129.45 (d, aryl), 128.73 (d, aryl), 128.20 (d, aryl), 59.35 (*C*H_2OC=OCH_3), 52.30 (OCH_3), 20.95 (OC=OCH_3, acetate).

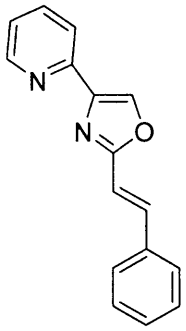
Acetic acid 2-carbamoyl-1-phenyl-allyl ester (92)



To a stirred solution of 2-(hydroxyphenylmethyl)-acrylamide **88** (0.20 g, 1.18 mmol) in pyridine (2 mL) was added acetic anhydride (0.14 mL, 1.35 mmol) at 20 °C. After 24 h the mixture was evaporated and the residue was purified by column chromatography (1:2 ethyl acetate: petroleum ether) to give **92** (0.15 g, 58 %) as a yellow oil; IR (thin film) (ν_{max}) 3384, 1664, 1595, 1450, 1031 cm^{-1}; Rf 0.2 (1:3 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.35 (5H, m, aryl), 6.84 (1H, s, C*H*OAc), 6.04 (1H, s, C=C*H*H), 5.86 (2H, bs, NH_2), 5.63 (1H, s, C=C*H*H), 2.09 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 169.57 (HN-C=O), 167.94 (O-C=O), 142.69 (*C*=CH_2), 137.45 (s, aryl), 128.66 (d, aryl), 128.47 (d, aryl), 127.08 (aryl), 122.47 (C=*C*H_2), 73.77 (*C*HOAc), 21.11 (OCH_3); LRMS m/z (EI) 219 (M^+, 2%), 202 (3%). 176 (100%), 159 (53%), 143 (2%), 132

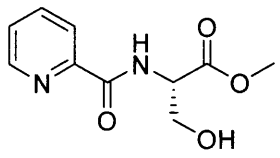
(23%), 115 (52%), 105 (84%), 91 (13%): HRMS calcd for $C_{12}H_{13}NO_3$ 219.0895. Found 219.0893.

2-(2-styryl-oxazol-4-yl pyridine) (96)



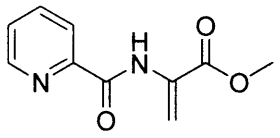
To a solution of 2-bromo-1-pyridin-2-yl-ethanone **81** (0.20 g, 0.99 mmol) in methanol (15 mL) was added cinnamamide (0.58 g, 3.99 mmol). The mixture was placed in the microwave (900 Watts) for 20 sec. The mixture was evaporated and the residue was dissolved in dichloromethane (30 mL), purified by column chromatography (1:4 ethyl acetate: petroleum ether) to give **96** (0.08 g, 35 %) as a colourless oil. Rf 0.2 (1:4 EtOAc: pet spt); IR (thin film) (ν_{max}) 3813, 1427, 1303, 1120, 1056, 693 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.59 (1H, ddd, *J*= 0.9, 1.68, 4.7 Hz, pyridyl), 8.24 (1H, s, oxazolyl), 7.91 (1H, ddd, *J*= 0.98, 2.0, 7.9 Hz, pyridyl), 7.77 (1H, ddd, *J*= 1.74, 7.3, 9.8 Hz, pyridyl) 7.54 (2H, m, aryl), 7.56 (1H, d, *J*=16.38 Hz, C=C*H*), 7.36 (3H, m, aryl), 7.22 (1H, ddd, *J*= 1.2, 4.8, 7.5 Hz, pyridyl), 6.97 (1H, d, *J*=16.38 Hz C=C*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 161.85 (s, pyridyl), 150.65 (s, oxazolyl), 149.52 (d, pyridyl), 142.25 (d, oxazolyl), 136.94 (d, oxazolyl), 136.84 (d, pyridyl), 136.43 (s, aryl), 135.40 (d, *C*=CH), 129.34 (d, aryl) 128.93 (d, aryl), 127.84 (d, aryl), 127.27 (d, pyridyl), 122.85 (d, C=*C*H), 120.23 (d, pyridyl); m/z +(CI) 249 (M+H, 2%), 214 (1%). 166 (4%), 148 (100%), 136 (2%): HRMS calcd for $C_{16}H_{12}N_2O$ [M+H] 249.1028. Found 249.1019.

3-Hydroxy-2-[(pyridine 2 carbonyl) amino] propionic acid methyl ester 98[6]



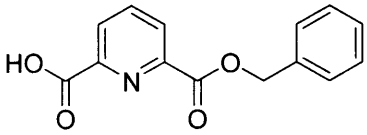
To a solution of propionic acid (3.0 g, 24.0 mmol) in anhydrous THF (80 mL) was added triethylamine (6.14 mL, 48.0 mmol). Isobutyl chloroformate (3.32 g, 24.0 mmol) was added dropwise to the mixture at -30 ℃. The reaction mixture was stirred over 2 h at -30 °C. Then L-serine methyl ester hydrochloride (7.46 g, 48.0 mmol) was added and the mixture was stirred at ambient temperature over 16 h. The mixture was evaporated and the residue was extracted with ethyl acetate (20 mL) and water (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (1:1 ethyl acetate: petroleum spirit) to give **98** (3.93 g, 72 %) as a colourless oil; Rf 0.25 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.83 (1H, bd, NH, *J*= 6 Hz), 8.59 (1H, ddd, *J*= 0.9, 1.67, 4.76 Hz, pyridyl), 8.17 (1H, , ddd, *J*= 1.0, 2.11, 7.8 Hz, pyridyl), 7.86 (1H, ddd, *J*= 1.72, 7.7, 9.4 Hz, pyridyl), 7.46 (1H, ddd, *J*= 1.24, 4.7, 7.6 Hz, pyridyl), 4.87 (1H, m, C*H*CH_2OH), 4.03 (2H, m CHC*H*$_2$OH), 3.82 (3H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 170.72 (H_3CO-*C*=O), 164.70 (HN-*C*=O), 149.10 (s, pyridyl), 148.31 (d, pyridyl), 137.49 (d, pyridyl), 126.60 (d, pyridyl), 122.47 (d, pyridyl), 63.55 (*C*H_2OH), 54.95 (*C*HCH$_2$OH), 52.84 (O-*C*H$_3$).

Methyl-2-(picolinamido)acrylate (99)



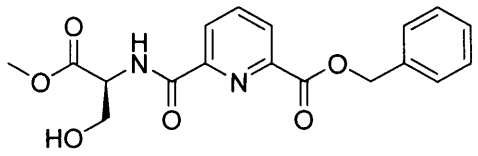
To a solution of 3-Hydroxy-2-[(pyridine 2 carbonyl) amino] propionic acid methyl ester **98** (3.00 g, 13.30 mmol) in dichloromethane (30 mL) was added triethylamine (1.80 mL, 14.5 mmol) and *p*-toluenesulfonyl chloride (2.50 g, 13.30 mmol). The mixture was stirred at 20 °C for 2 h. The mixture was evaporated and the residue was purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give **99** (2.20 g, 82 %) as an orange oil; IR(thin film) ν_{max} (cm^{-1}) 3438, 1628, 1525 1126; Rf 0.5 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.61 (1H, ddd, *J*= 0.9, 1.48, 4.77 Hz, pyridyl), 8.19 (1H, ddd, *J*= 0.9, 2.09, 7.78 Hz, pyridyl), 7.86 (1H, ddd, *J*= 1.67, 7.6, 9.3 Hz, pyridyl), 7.48 (1H, ddd, *J*= 1.2, 4.78, 7.6 Hz, pyridyl), 6.81 (1H, s, C=C*H*), 6.01 (1H, s C=C*H*), 3.85 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 164.37 (H_3CO-*C*=O), 163.01 (HN-*C*=O), 149.47 (s, pyridyl), 148.35 (d, pyridyl), 137.52 (d, pyridyl), 131.19 (*C*=CH_2), 126.58 (d, pyridyl), 122.22 (d, pyridyl), 109.18 (C=*C*H_2), 52.95 (O-*C*H_3); LRMS m/z (EI) 207 (M+H, 100%), 191 (7%), 177 (3%), 147 (4%), 96 (11%); HRMS calcd for $C_{10}H_{10}N_2O_3$ 207.0770. Found 207.0801.

2,6-Pyridinedicarboxylic acid monobenzyl ester (101)[7,8]



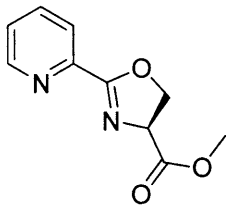
A mixture of pyridine-2,6-dicarboxylic acid (2.00 g, 1.2 mmol), water (4.8 mL), benzyl alcohol (13.8 mL, 130 mmol) and concentrated sulfuric acid (0.66 mL) was heated at reflux for 2 h, and then allowed to stir at 25 °C for 16 h. The mixture was neutralised with saturated aqueous sodium hydrogen carbonate (170 mL) and extracted with chloroform (150 mL) to remove the dibenzyl ester (20%). The aqueous layer was acidified to pH 2.0, at which point the monoester crystallized as white needles. Additional monoester was obtained by extracting the resulting mother liquor with chloroform to give **101** as white needles (1.9 g, 61%). mp 130-132 °C, lit.[8] mp 132-133 °C ; Rf 0.2 (1:4 EtOAc: petroleum ether); ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.21 (3H, m, pyridyl), 7.37 (5H, m, aryl), 5.40 (2H, m, OC*H*$_2$), 4.12 (1H, bs, O*H*); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_c 165.52 (HO-*C*=O), 164.01 (CH_2O-*C*=O), 148.05 (s, pyridyl), 147.52 (s, aryl), 139.15 (s, pyridyl), 128.46 (d, pyridyl), 128.27 (d, pyridyl), 128.15 (d, pyridyl), 127.95 (d, aryl), 127.87 (d, aryl), 127.43 (d, aryl), 66.81 (OCH_2).

(*S*)-6-(2-Hydroxy-1-methoxycarbonyl-ethyl carbamoyl)-pyridine-2-carboxylic acid benzyl ester (102)



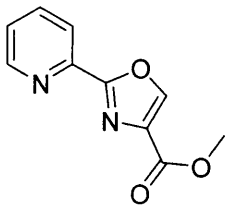
Isobutyl chloroformate (0.28 mL, 2.14 mmol) was added to a solution of pyridine 2,6-dicarboxylic acid monobenzyl ester **101** (0.55 g, 2.14 mmol) and triethylamine (0.6 ml, 4.28 mmol) in dichloromethane (40 mL) at -30 °C. After 1 h, L-serine methyl ester hydrochloride (0.67 g, 4.28 mmol) was added in portion over 10 min. The mixture was left to stir and warm up from -20 °C to ambient temperature over 16 h. Then dichloromethane (30 mL) was added and the mixture was extracted with water (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to leave a residue which was purified via column chromatography (1:1 ethyl acetate: petroleum ether) to give **102** (0.40 g, 52 %) as white plates, mp 88-90 °C; IR (KBr) ν_{max} 3525, 3269, 1741, 1687, 1539, 1045 cm^{-1}; Rf 0.2 (1:1 EtOAc: petroleum ether); ^1H NMR ($CDCl_3$, 300 MHz) δ_H 9.04 (1H, d, *J*= 3Hz, N*H*), 8.29 (1H, d, *J*= 6Hz, pyridyl), 8.22 (1H, d, *J*= 6Hz, pyridyl), 8.17 (1H, t, *J*= 7.8 Hz, pyridyl), 7.45 (5H, m, aryl), 5.41 (2H, s, O*CH*$_2$), 4.84 (1H, m, C*H*), 4.01 (2H, m, C*H*$_2$OH), 3.78 (3H, s, O*CH*$_3$);^{13}C NMR ($CDCl_3$, 75 MHz) 170.51 (HN-*C*=O), 164.36 (CH_3O-*C*=O), 163.84 (CH_2O-*C*=O), 149.72 (s, pyridyl), 146.52 (s, aryl), 138.63 (s, pyridyl), 135.23 (d, pyridyl), 128.74 (d, pyridyl), 128.57 (d, pyridyl), 128.31 (d, aryl), 127.57 (d, aryl), 125.74 (d, aryl), 67.77 (O*C*H$_2$), 63.17 (*C*H$_2$-OH), 55.23 (HOCH$_2$-*C*H), 52.73 (O*C*H$_3$). LRMS m/z +(FAB) 381 (M+Na, 100%), 359 (92%), 338 (42%), 321 (6%), 269 (3%), 235 (2%): HRMS calcd for $C_{18}H_{28}N_2O_6$ [M+Na] 381.1063. Found 381.1069. Calculated for $C_{18}H_{18}N_2O_6$ C, 60.33, H, 5.06, N 7.82%. Found C, 60.09, H, 5.17, N, 7.45%.

2-Pyridin-2-yl- 4, 5-dihydro-oxazole-4-carboxylic acid methyl ester 104



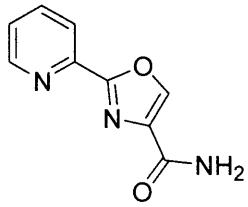
3-Hydroxy-2-[(pyridine 2 carbonyl) amino] propionic acid methyl ester **98** (1.0 g, 4.5 mmol) placed under an argon atmosphere and anhydrous dichloromethane (20 mL) was then added. The temperature of the reaction was reduced to -78 °C; DAST (0.63 mL, 4.8 mmol) was then added dropwise (forming a yellow solution). The mixture was left to stir at -78 °C for 2 h. Potassium carbonate (1.20 g, 9.01 mmol) was then added to the mixture, which was left to stir to ambient temperature for 16 h. The mixture was then poured into a solution of sodium carbonate (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (3:1 hexanes: ethyl acetate) to give **104** (0.66 g, 72 %) as a colourless oil; $[\alpha_D]^{23}$ = -12 ° (*c*= 1.0, $CHCl_3$); IR (thin film) ν_{max} (cm^{-1}) 3411, 2916, 1737, 1639, 1045, 966; Rf 0.35 (EtOAc); ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.67 (1H, ddd, *J*= 0.9, 1.7, 4.78 Hz, pyridyl), 8.06 (1H, ddd, *J*= 1.0, 2.0, 7.8 Hz, pyridyl), 7.76 (1H, ddd, *J*= 1.72, 7.7, 9.4 Hz, pyridyl), 7.40 (1H, ddd, *J*= 1.2, 4.8, 7.6 Hz, pyridyl), 4.98 (1H, m, C*H*, oxazolinyl), 4.68 (1H, m, CH_2, oxazolinyl), 3.84 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 171.18 (H_3CO-*C*=O), 165.34 (s, O-*C*=N), 149.80 (s, pyridyl), 145.96 (d, pyridyl), 136.73 (d, pyridyl), 126.05 (d, pyridyl), 124.41 (d, pyridyl), 70.17 (t, *C*H_2 oxazolinyl), 68.70 (d, *C*HCH_2, oxazolinyl), 52.78 (O-*C*H_3); LRMS m/z (EI) 207 (M+H, 4%), 193 (2%), 147 (100%), 119 (24%), 106 (8%), 92 (68%), 78 (47%): HRMS calcd for $C_{10}H_{10}N_2O_3$ 206.06914. Found 206.06916.

2- Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester (100)



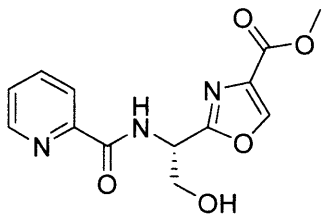
To a solution of 2-pyridin-2-yl-4,5-dihydro-oxazole-4-carboxylic acid methyl ester **104** (0.15 g, 0.727 mmol) in dichloromethane (10 mL) was added DBU (0.29 mL, 1.45 mmol). The mixture was left to stir for 5 min at -10 °C. To the mixture was added bromotrichloromethane (0.10 mL, 0.75 mmol). The mixture was allowed to warm up with stirring from -10°C to ambient temperature over 20 h. Saturated aqueous ammonium chloride (20 mL) was added and the organic layer was isolated, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (ethyl acetate) to give **100** (0.125 g, 85%) as a beige solid, mp 124.5-126.5 °C; IR (KBr) ν_{max} 3415, 2925, 1711, 1633, 1047, 999 cm^{-1}; Rf 0.50 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.74 (1H, ddd, *J*= 0.9, 1.7, 4.8 Hz, pyridyl), 8.38 (1H, s, oxazole), 8.28 (1H, ddd, *J*= 1.0, 2.0, 7.8 Hz, pyridyl), 7.86 (1H, ddd, *J*= 1.7, 7.8, 9.3 Hz, pyridyl), 7.44 (1H, ddd, *J*= 1.1, 4.78, 7.6 Hz, pyridyl), 3.96 (3H, s, OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 164.66 (CH_3O-*C*=O), 161.50 (s, pyridyl), 150.01 (C=N, oxazolyl), 145.12 (d, pyridyl), 144.91 (C=*C*H, oxazolyl), 137.26 (*C*=CH, oxazolyl), 134.60 (d, pyridyl), 125.53 (d, pyridyl), 122.89 (d, pyridyl), 52.38 (OCH_3): HRMS calcd for $C_{10}H_8N_2O_3$ 204.05349. Found 204.05367: *Anal.* Calcd for $C_{10}H_8N_2O_3$: C, 58.82; H, 3.95; N, 13.72. Found: C, 58.64; H, 3.92; N, 13.50.

2-Pyridin-2-yl-oxazole-4-carboxylic acid amide (77)



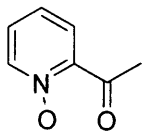
2-Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester **100** (0.10 g, 0.48 mmol) was dissolved in 0.880 aqueous ammonia (4 mL) and dioxane (1 mL). The mixture was left to stir for 30 h at ambient temperature. The precipitate was filtered and washed with water to leave **77** (0.07 g, 79 %) as a beige crystalline solid, mp 189 °C; IR (KBr) (ν_{max}) 3398, 2925, 1638, 1061, 968 cm^{-1}; Rf baseline (EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 8.73 (1H, s, oxazolyl), 8.73 (1H, ddd, *J*= 1.0, 1.7, 4.8 Hz, pyridyl), 8.13 (1H, ddd, *J*= 1.0, 2.0, 7.8 Hz, pyridyl), 8.02 (1H, ddd, *J*= 1.72, 7.9, 9.3 Hz, pyridyl), 7.78 (1H, bs, NH), 7.61 (1H, bs, NH), 7.57 (1H, ddd, *J*= 1.1, 4.8, 7.5 Hz, pyridyl); ^{13}C NMR (75 MHz, DMSO-d_6) δ_c 161.59 (H_2N-*C*=O), 160.34 (s, pyridyl), 149.97 (C=N, oxazolyl), 144.83 (d, pyridyl), 143.01 (d, C=*C*H, oxazolyl), 137.60 (*C*=CH, oxazolyl), 134.68 (d, pyridyl), 125.58 (d, pyridyl), 122.28 (d, pyridyl). *Anal.* Calcd for $C_9H_7N_3O_2$: C, 57.43; H, 3.88; N, 21.48. Found: C, 56.99; H, 3.80; N, 21.36.

Methyl-2-(2-hydroxyl-1-(picolinamido) ethyl)oxazoles-4-carboxylate (106)



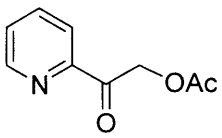
To a solution of picolinic acid (0.20 g, 1.62 mmol) in DMF (20 mL) was added BOP (0.80 g, 1.78 mmol), DIEA (0.68 mL, 3.72 mmol) and HOBT (0.24 g, 1.78 mmol). The mixture was stirred for 1.5 h at 20 °C, then the temperature was lowered to -62 °C. A solution of 2-(1-amino-2-hydroxyl-ethyl)-oxazole-4-carboxylic acid methyl ester **105** (0.29 g, 1.55 mmol) in DMF (10 mL) was added to the mixture dropwise. The solution was stirred for 24 h at -62 °C, then ethyl acetate (50 mL) was added. The organic layer was washed with water (3x 50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **106** (0.18 g, 40%) as a brown oil; $[\alpha_D]^{23}$= -27.3 ° (*c*=0.76, $CHCl_3$); IR (thin layer) (ν_{max}) 3436, 1662, 1265, 1095 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ_H 8.66 (1H, m, pyridyl), 8.52 (1H, s, oxazolyl), 8.09 (1H, m, pyridyl), 7.96 (1H, m, pyridyl), 7.58 (1H, m, pyridyl), 5.38 (1H, m C*H*CH_2), 4.08 (2H, m, C*H*$_2$OH), 3.86 (3H, s, OC*H*$_3$); ^{13}C NMR (100 MHz, CD_3OD) δ_c 166.56 (HNC=O), 164.70 (OC=O), 162.93 (s, pyridyl), 150.37 (s, oxazolyl), 149.88 (d, pyridyl), 146.46 (d, oxazolyl), 138.86 (d, pyridyl), 134.19 (d, oxazolyl), 128.14 (d, pyridyl), 123.33 (d, pyridyl), 63.38 (C*H*$_2$OH), 52.57 (C*H*CH_2OH), 51.38 (OCH_3); LRMS m/z + (FAB) 219 (M+Na, 100%), 221 (9%). 199 (31%), 177 (96%): HRMS calcd for $C_{13}H_{13}N_3O_5$ [M+Na] 314.0752. Found 314.0758.

2-Acetylpyridine 1-oxide (109)[9]



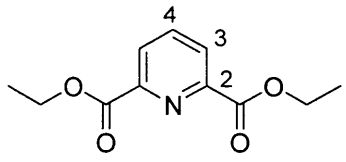
To a solution of 2-acetylpyridine (6.05 g, 49.9 mmol) in chloroform (50 mL) was added *m*-CPBA (11.0 g, 64.9 mmol) and stirred at 20 °C for 0.5 h then heated at reflux for a further 0.5 h. The mixture was evaporated and the residue was dissolved in ether (50 mL) and water (50 mL). The organic layer was washed water (2x50 mL), the water washings was evaporated to leave **109** (6.7 g, 98%) as a brown residue. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.53 (1H, dd, *J*=2.2, 7.8 Hz, pyridyl), 7.40 (1H, m, pyridyl), 7.17 (2H, m, pyridyl), 2.52 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 194.86 (C=O), 146.60 (s, pyridyl), 140.42 (d, pyridyl), 128.04 (d, pyridyl), 126.48 (d, pyridyl), 125.42 (d, pyridyl), 30.39 (CH_3).

Acetoxymethyl 2-pyridyl ketone (110)[9]



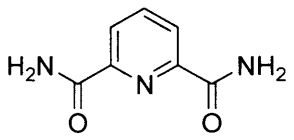
A solution of 2-acetylpyridine 1-oxide **109** (2.44 g, 18.0 mmol) in acetic anhydride (35 mL) was heated at 140 °C for 1.5 h. The mixture was evaporated and the residue was purified by column chromatography (4:1, 40-60 °C petroleum ether: ethyl acetate). The mixture was evaporated to leave **110** (1.02 g, 32%) as a yellow oil. Rf 0.2 ($CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.96 (1H, ddd, *J*= 0.9, 1.73, 4.81 Hz, pyridyl), 8.03 (1H, ddd, *J*= 1.0, 2.2, 7.8 Hz, pyridyl), 7.83 (1H, ddd, *J*= 1.7, 7.68, 9.3 Hz, pyridyl), 7.51 (1H, ddd, *J*= 1.0, 4.8, 7.7 Hz, pyridyl), 5.62 (2H, s, OCH_2), 2.21 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 175.98 (C=O, ketone), 171.71 (C=O, acetate), 170.97 (C=O, ester), 146.49 (s, pyridyl), 137.44 (d, pyridyl), 123.28 (d, pyridyl), 123.07 (d, pyridyl), 121.12 (d, pyridyl) 65.85 (CH_2), 21.73 (CH_3).

Pyrdine-2,6-dicarboxylic acid dimethyl ester (122)[10]



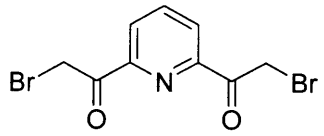
2,6-Dipicolinic acid (2.00 g, 12 mmol) and thionyl chloride (11.7 mL, 130 mmol) was heated at reflux for 2 h. The excess thionyl chloride was evaporated, and the residue dissolved in dry benzene (13.3 mL). After the solution had been stirred at 0 °C for 15 min, ethanol (4.0 mL) was added dropwise. The mixture was then heated at reflux for 2 h, cooled to 25 °C, and aqueous 20% sodium carbonate (20 mL) was added, the mixture was then left to stir for 10 min. The organic layer was collected and the aqueous layer extracted with diethyl ether (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, and evaporated to give **122** (2.37 g, 89%) as a pale yellow oil: Rf 0.2 (1:1 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.27 (2H, d, *J*=7.8 Hz, 3-H, 5-H), 7.99 (1H, t, *J*=8.1 Hz, 4-H), 4.47 (4H, q, *J*=6.3, 9 Hz, 2 x CH_2), 1.43 (6H, m, 2 x CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 164.49 (*C*=O), 148.49 (s, pyridyl), 138.26 (d, pyridyl), 128.76 (d, pyridyl), 62.20 (O*C*H_2), 15.17 (CH_3).

Pyridine-2,6-dicarboxamide (120)[10]



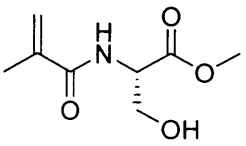
To pyridine-2,6-dicarboxylic acid diethyl ester **122** (2.00 g, 8.96 mmol) was added concentrated aqueous ammonia (0.880, 7.0 mL) and the resulting white suspension was stirred 16 h. The white solid formed was collected, washed with water to give **120** (0.75 g, 51%) as white platelets; mp 298 °C (dec), lit mp[10] 317 °C; Rf baseline (EtOAc); ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.86 (2H, bs, N-H), 8.14 (3H, m, pyridyl), 7.70 (2H, bs, N-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_c 165.27 (C=O), 148.98 (s, pyridyl), 139.08 (d, pyridyl), 124.07 (d, pyridyl).

1, 1 –(Pyridine-2,6-diyl)bis(2-bromoethanone) (123)[11]



To a solution of the 1,1-(pyridine-2,6-diyl)bisethanone (**236**) (1.25 g, 7.66 mmol) in chloroform (50 mL) was added bromine (0.87 mL, 16.9 mmol) dropwise. The mixture was heated at reflux for 16 h. Evaporation to afford **123** (2.4 g, 98 %) as a brown solid, mp 159-161°C, lit.[11] mp160-162 °C; Rf 0.2 (1:2 EtOAc: petroleum ether); ^{1}H NMR (300MHz, $CDCl_3$) δ_H 8.33 (2H, d, *J*=7.8 Hz, pyridyl*)*, 8.21 (1H, t, *J*=9 Hz, pyridyl*)*, 4.61 (4H, s, 2CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 191.36 ($BrCH_2$-*C*=O), 154.21 (s, pyridyl), 138.41 (d, pyridyl) 126.67 (d, pyridyl), 39.10 (*C*H_2Br).

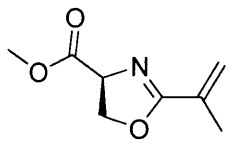
3-Hydroxy-2-(2-methacryloylamino)-propionic acid methyl ester (127)



To a solution of methacrylic acid (0.36 g, 3.79 mmol) in anhydrous THF (20 mL) under an argon atmosphere was added triethylamine (0.99 mL, 7.58 mmol). The ice bath temperature was lowered to -30 ℃, then isobutyl chloroformate (0.49 mL, 3.79 mol) was added. The mixture was left to stir at -30 ℃ for 2 h. Then L-serine methyl ester hydrochloride (0.59 g, 3.79 mmol) was added in one portion. The thick slurry was stirred at -30 ℃ then allowed to warm from -30 ℃ to ambient temperature for 16 h. The mixture was evaporated and the residue was extracted with ethyl acetate (30 mL) and water (30 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography (5:1 petroleum ether: ethyl acetate) to give **127** (0.32 g, 45%) as a colourless oil; $[\alpha_D]^{23}$ = -9.0 ° (c 1.0 in $CHCl_3$); IR (thin film) ν_{max} 3364, 2852, 1733, 1661,1520, 1079, 935 cm^{-1}; Rf 0.2 (1:5 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.81 (1H, bs, NH,), 5.80 (1H, s, C=C*H*), 5.41 (1H, s, C=C*H*), 4.72 (1H, m, CH_2CH), 3.95 (2H, m, CH_2OH), 3.80 (3H, s, OCH_3),

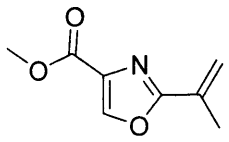
1.98 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 171.08 (HN-*C*=O), 168.65 (CH_3O-*C*=O), 139.04 (C=*C*-C=O), 121.08 (*C*=C-C=O), 63.37 (*C*H_2OH), 54.90 (H*C*-CH_2OH), 52.87 (O-*C*H_3), 18.48 (*C*H_3); LRMS m/z (EI): 188 (M+H, 100%), 170 (74%), 157 (7%), 141 (2%), 128 (13%), 97 (11%): HRMS calcd for $C_8H_{13}NO_4$ 187.08445, found 187.08527.

2-Isoprenyl-4, 5-dihydro-oxazole-4-carboxylic acid methyl ester (128)



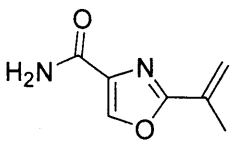
To a solution of amide **127** (0.26 g, 1.37 mmol) under an argon atmosphere was added anhydrous dichloromethane (10 mL). The temperature of the reaction was lowered to -78 ℃. Then DAST (0.29 mL, 2.20 mmol) was added dropwise (forming a light yellow solution). The mixture was stirred at -78 °C for 6 h. Potassium carbonate (0.35 g, 2.6 mmol) was then added and the stirred mixture was allowed to warm from -78 °C to ambient temperature over 16 h. The mixture was then poured into aqueous sodium carbonate (20 mL). The organic layer was isolated, and dried over $MgSO_4$, filtered and evaporated. The residue which was purified by column chromatography (3:1, petroleum ether: ethyl acetate) to give **128** (0.16 g, 82%) as a colourless oil; $[\alpha_D]^{23}$= 6.8° (*c*=1.0, $CHCl_3$); IR (thin film) ν_{max} 3416, 2963, 1743, 1608,1441, 1066, 989 cm^{-1}; Rf 0.2 (1:3 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 5.80 (1H, s, C=C*H*), 5.39 (1H, s, C=C*H*), 4.83 (1H, m, CH_2C*H*), 4.47 (2H, m, CH_2, oxazolinyl), 3.77 (3H, s, OCH_3), 2.01 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 171.58 (*C*=O), 167.08 (C=N, oxazolinyl) 132.28 (*C*=CH_2), 123.12 (C=*C*H_2), 69.30 (CH*C*H_2, oxazolinyl), 68.68 (*C*HCH_2, oxazolinyl), 52.64 (O*C*H_3), 19.28 (*C*H_3); LRMS m/z (CI): 169 (M^+, 94%), 145 (100%), 131 (6%), 117 (23%), 101 (33%), 96 (11%): HRMS calcd for $C_8H_{11}NO_3$ [M+H] 170.08171, found 170.08172.

2-Isoprenyl-oxazole-4-carboxylic acid methyl ester (129)



To a solution of oxazoline **128** (0.13 g, 0.77 mmol) in dichloromethane (10 mL) was added DBU (0.22 mL, 1.5 mmol). The temperature was then lowered to -10 °C then bromotrichloromethane (0.08 mL, 0.81 mmol) was added. The mixture was allowed to warm up with stirring from -10 °C to ambient temperature over 16 h. The mixture was evaporated to leave a brown residue which was purified by column chromatography (ethyl acetate) to leave **129** (0.10 g, 85%) as a yellow oil; IR (thin film) ν_{max} 3414, 2933, 1726, 1572, 1438, 1004, 933 cm^{-1}; Rf 0.2 (1:2 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.23 (1H, s, oxazolyl), 6.03 (1H, s, C=C*H*), 5.41 (1H, s, C=C*H*), 3.87 (3H, s, OCH_3), 2.19 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 163.30 (CH_3O-*C*=O), 161.34 (*C*=N, oxazolyl), 143.71 (*C*=CH, oxazolyl), 134.09 (C=*C*H, oxazolyl), 131.13 (C=*C*H_2), 120.08 (C=*C*H_2), 52.21 (O*C*H_3), 19.00 (*C*H_3); LRMS m/z (EI): 169 (M+H, 94%), 170 (32%), 157 (5%), 136 (36%), 110 (100%), 86 (48%): HRMS calcd for $C_8H_9NO_3$ 169.0738, found 169.0740.

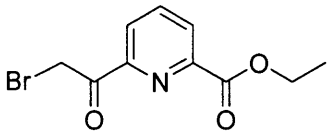
2-Isoprenyl-oxazole-4-carboxylic acid amide (130)



The ester **129** (0.09 g, 0.53 mmol) was dissolved in 0.880 aqueous ammonia (2 mL) and dioxane (1 mL). The mixture was left to stir for 30 h at room

temperature. The reaction mixture was then evaporated and the residue was extracted with ethyl acetate (15 mL) and water (15 mL). The organic layer was dried over $MgSO_4$, filtered to give **130** (0.06 g, 75 %) as a beige foam; IR (thin film) ν_{max} 3383, 2922, 1464, 1377 cm^{-1}; Rf baseline (EtOAc); ^1H NMR (300 MHz, $CDCl_3$) δ_H 8.16 (1H, s, oxazolyl), 6.90 (1H, bs, NH), 6.53 (1H, bs, NH), 5.96 (1H, s, C=C*H*), 5.35 (1H, s, C=C*H*), 2.14 (3H, s, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 163.22 (NH_2C=O), 162.36 (*C*=N, oxazolyl), 141.30 (*C*=CH), 136.54 (C=*C*H, oxazolyl), 131.04 (*C*=CH, oxazolyl), 119.66 (C=*C*H_2), 18.82 (CH_3); LRMS m/z (EI): 152 (M^+, 100%), 136 (22%), 124 (7%), 108 (9%), 85 (83%), 69 (48%); HRMS calcd for $C_7H_8N_2O_2$ 152.0530, found 152.0534.

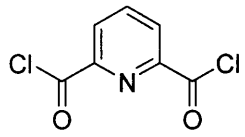
6-(2-Bromoacetyl)-pyridine-2-carboxylic acid ethyl ester (131)



To a suspension of sodium methoxide (0.39 g, 7.30 mmol) in benzene (8.5 mL), ethyl acetate (1.25 mL) was added pyrdine-2,6-dicarboxylic acid diethyl ester **122** (0.50 g, 2.23 mmol). After 10 min the reaction was cooled and then 10 M hydrochloric acid (2.23 mL) was added dropwise. Then the suspension was heated at reflux for 16 h, then the organic layer was separated and discarded. The aqueous layer was neutralised using solid anhydrous sodium carbonate. Ether (20 mL) was added and then the organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was then dissolved in chloroform (20 mL) and bromine (0.07 mL) was added dropwise. The reaction was stirred at reflux for 3 h. The mixture was then evaporated down and then purified by column chromatography (dichloromethane) to afford **131** (0.24 g, 64 %) as a brown oil; IR ν_{max} 2930, 1708, 1456, 1377, 761 cm^{-1}; Rf 0.2 (DCM); ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.22 (2H, d, *J*=6 Hz, pyridyl), 8.18 (1H, t, *J*=8.8 Hz, pyridyl), 4.78 (2H, m, CH_2O), 4.47 (2H, s, CH_2Br), 1.23 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_c 191.36 ($BrCH_2OC$=O), 163.35 (CH_3CH_2O-*C*=O), 150.34 (s, pyridyl), 149.49 (s, pyridyl), 147.80 (d, pyridyl), 129.28 (d, pyridyl), 128.39 (d, pyridyl), 127.40 (d, pyridyl), 63.16 (*C*H_2O), 39.49 (*C*H_2Br), 14.29 (*C*H_3CH_2); LRMS m/z

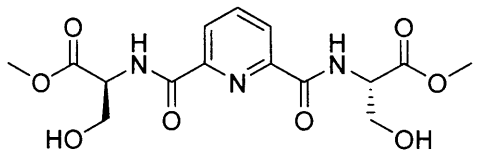
(EI): 272 (M^{81+}, 60%), 270 (M^{79+}, 62%), 242 (54%), 226 (100%), 178 (61%): HRMS calcd for $C_{10}H_{10}BrNO_3$ 270.9844, found 270.9857.

Pyridine-2,6-dicarbonyl dichloride (235)[10]



To a stirred solution of pyridine-2,6-dicarboxylic acid (1.00 g, 5.86 mmol) and thionyl chloride (2.14 mL, 30 mmol) at 25 °C was added two drops of DMF. The mixture was then heated at reflux for 2 h. The mixture was cooled to 25 °C and excess thionyl chloride was evaporated to afford **235** (2.77 g, 77%) as an oil that was used without further purification.

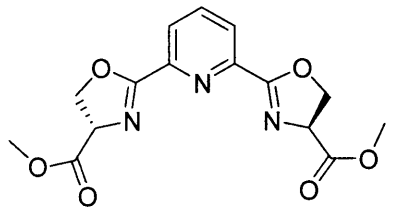
3- Hydroxy-2-{[6-(2-hydroxy-1-methoxycarbonyl-ether carbamoyl)-pyridine-2-carbonyl]-amino}-propionic acid methyl ester (136)[12]



To a solution of L-serine methyl ester hydrochloride (2.04 g, 13.9 mmol) in chloroform (20 mL) was added triethylamine (4.02 mL, 29.3 mmol) at ambient temperature. To the mixture was added pyridine-2,6-dicarbonyl dichloride (**235**) (1.21 g, 5.96 mmol) in chloroform (10 mL). The resulting mixture was stirred for 24 h at room temperature. The white solid was filtered off and the filtrate was evaporated and the residue was purified by column chromatography (20:1 ethyl acetate: methanol) to give **136** (1.32 g, 64 %) as white flakes, mp 165.0-168.0°C, lit.[12] mp 165.0-167.0 °C; Rf 0.2 (1:1 EtOAc: petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.85 (2H, d, NH, J=7.9 Hz), 8.13 (2H, d, J =7.8 Hz, pyridyl), 7.82 (1H, t, J =7.8 Hz, pyridyl), 4.84 (2H, m, C*H*CH_2OH), 4.22 (4H, m CH_2OH), 3.83 (6H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 171.21 (HN-*C*=O),

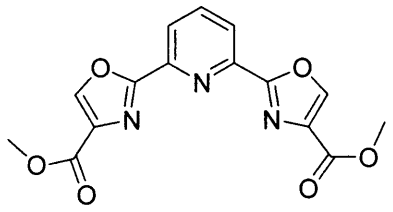
167.92 (O-*C*=O), 148.17 (s, pyridyl), 138.86 (d, pyridyl), 125.32 (d, pyridyl), 62.90 (*C*H_2OH), 55.34 (H*C*-CH_2OH), 53.01 (O-*C*H_3).

Pyridine-2,6-bisoxazozaline methyl ester 137[12]



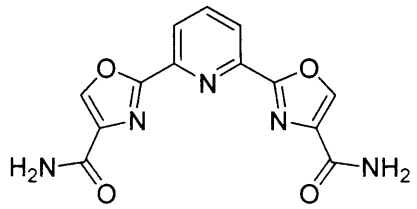
3-Hydroxy-2-{[6-(2-hydroxy-1-methoxycarbonyl-ether carbamoyl)-pyridine-2-carbonyl]-amino}-propionic acid methyl ester (**136**) (0.50 g, 1.26 mmol) was placed under argon atmosphere and anhydrous dichloromethane (10 mL) was added. The temperature of the mixture was lowered to -78 °C; DAST (0.37 mL, 2.77 mmol) was then added dropwise over 5 min. The mixture was left to stir at 78 °C for 2 h. Potassium carbonate (0.195 g, 1.41 mmol) was then added to the mixture which was left to stir from -78 °C to ambient temperature. The mixture was then poured into saturated aqueous sodium hydrogen carbonate (20 mL). The organic layer was dried with $MgSO_4$, filtered and evaporated to give **137** (0.40 g, 90 %) as a yellow oil; Rf 0.2 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.17 (2H, d, *J*=7.7 Hz, pyridine), 7.89 (1H, t, *J* =7.8 Hz, pyridine), 5.01 (2H, m, C*H* oxazolinyl), 4.69 (4H, m CH_2 oxazolinyl), 3.80 (6H, s, OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ_c 171.06 (H_3CO-*C*=O), 164.85 (s, O-*C*=N, oxazolinyl), 146.17 (s, pyridyl), 137.58 (d, pyridyl), 126.74 (d, pyridyl), 70.43 (*C*H_2, oxazolinyl), 68.67 (s, OCH-CH_2, oxazolinyl), 52.85 (O-*C*H_3).

Pyridine-2,6-bisoxazole methyl ester 138



To a solution of pyridine-2,6-bisoxazozaline methyl ester **137** (0.20 g, 6.0 mmol) in dichloromethane (8 mL) was added DBU (0.35 g, 2.44 mmol). After stirring for 10 min bromotrichloromethane (0.13 mL, 1.22 mmol) was added at -10°C to the mixture and stirred over 20 h. The mixture was evaporated to leave a residue that was purified by column chromatography (ethyl acetate) to give **138** (0.15 g, 80 %) as a white solid; mp 158.0-160.0 °C; IR (KBr) ν_{max} 3429, 1747, 1574, 1325, 1155, 1107 cm^{-1}; Rf 0.2 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.40 (2H, s, oxazolyl), 8.29 (2H, d, *J*=7.9 Hz pyridyl), 8.02 (1H, t, *J*=7.8 Hz, pyridyl), 3.97 (6H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 161.32 (H_3CO-*C*=O), 160.26 (s, pyridyl), 145.51 (C=N, oxazolyl), 145.31 (C=*C*H, oxazolyl), 138.42 (d, pyridyl), 134.73 (C=*C*H, oxazolyl), 124.44 (d, pyridyl), 52.41 (O-*C*H_3); Calculated for $C_{15}H_{11}N_3O_6$ C, 54.72, H, 3.37, N 12.76% Found C, 54.37, H, 3.34, N, 12.54%.

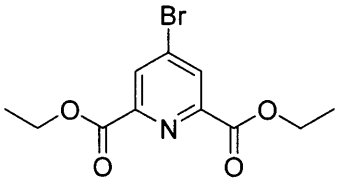
2,6-Pyridin-2-yl-oxazole-4-carboxylic acid amide 115



2,6-Pyridin-2-yl-oxazole-4-carboxylic acid methyl ester (**138**) (0.20 g, 0.60 mmol) was dissolved in 0.880 aqueous ammonia (4 mL) and dioxane (1 mL). The reaction was stirred for 30 h at room temperature. The precipitate was filtered and washed with water to give **115** (0.08 g, 88 %) as a white solid, mp 268 °C (dec); IR (KBr) ν_{max} 3506, 2324, 1638, 1271, 1066 cm^{-1}; Rf baseline (EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 8.81 (2H, s, oxazolyl), 8.27 (3H, m,

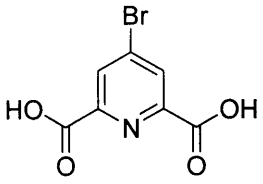
pyridyl), 7.83 (2H, bs, NH), 7.63 (2H, bs, NH), 7.57 (1H, m, pyridyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ_c 161.54 (H_2N-*C*=O), 158.92 (s, O-*C*=N, oxazolyl), 145.25 (s, pyridyl), 143.49 (s, *C*=CH, oxazolyl), 139.38 (d, C=*C*H oxazolyl), 137.78 (d, pyridyl), 123.85 (d, pyridyl); LRMS m/z (FAB): 322 (M+Na, 34%), 300 (57%), 273 (13%), 245 (24%), 176 (100%); HRMS calcd for $C_{13}H_9N_5O_4$ [M+Na] 322.0552, found 322.0533.

4-Bromopyridine-2,6-dicarboxylic acid diethyl ester (167)[13]



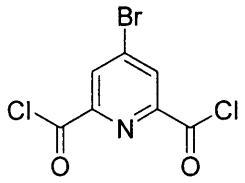
Chelidamic acid monohydrate **(168)** (0.20 g, 1.1 mmol) and phosphorus pentabromide (3.05 g, 7.1 mmol) were heated to 90 °C whereupon it formed a melt. The mixture was then stirred at 120 °C at reflux for 16 h. After the mixture was cooled chloroform (20 mL) was added and the mixture was filtered. The filtrate was cooled to 0 °C and then ethanol (16 mL) was added dropwise. The mixture was heated at reflux for 3 h. The mixture was evaporated and the residue was purified by column chromatography (1: 1 ethyl acetate: 40-60 °C petroleum ether), to give **167** (0.26 g, 80%) as yellow semi-solid; Rf 0.5 (EtOAc) ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.42 (2H, s, pyridyl), 4.48 (4H, q, *J*= 6.0, 15Hz, CH_2CH_3), 1.45 (6H, t, *J*= 6.0 Hz, CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_c 163.55 (C=O), 149.53 (s, pyridyl), 136.50 (s, pyridyl, *C*-Br), 131.07 (d, pyridyl), 62.74 (OCH_2CH_3), 14.20 (OCH_2CH_3).

4-Bromopyridine-2, 6-dicarboxylic acid (166)[13]



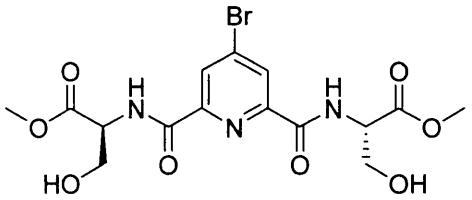
To a solution of the diester **167** (0.19 g, 0.63 mmol) in THF (5 mL) was added a solution of lithium hydroxide monohydrate (0.06 g, 1.5 mmol) in water (0.83 mL). The mixture was stirred for 17 h at 38 °C. Then the solution was acidified to pH 1 with 3M HCl. The mixture was evaporated and the residue was dissolved in ethyl acetate and filtered. The filtrate was evaporated to give **166** (0.12 g, 78%) as a beige solid, mp 206 °C (dec), lit.[13] mp 205-207 °C (dec); Rf baseline (EtOAc); ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 8.35 (2H, s, pyridyl; ^{13}C NMR (DMSO-d_6, 300 MHz) δ_c 165.15 (C=O), 152.39 (s, pyridyl), 136.86 (s, pyridyl, C-Br), 128.29 (d, pyridyl).

4-Bromo-pyridine-2,6-dicarbonyl dichloride (169)[13]



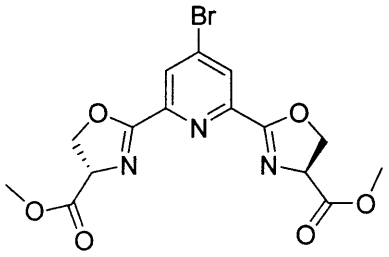
Thionyl chloride (1.39 mL, 19.0 mmol), dimethylformide (3 drops) were added to **166** (0.40 g, 1.62 mmol) and stirred at reflux for 2 h. Thionyl chloride was evaporated off using the rotary evaporator. To leave **169** as an orange oil (0.46 g, quantitative yield).

S-2-{[4-Bromo-6-(2-hydroxy-1-methoxycarbonyl-ethylcarbomyl)-pyridine-2-carbonyl]-amino}3-hydroxy-propionic acid methylester (165)



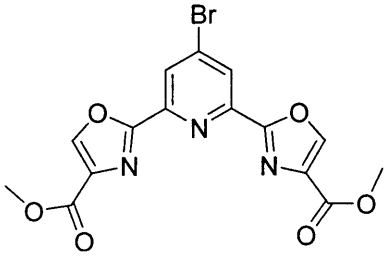
To a stirred suspension of L-serine methyl ester (0.63 g, 4.06 mmol) in chloroform (50 mL) was added triethylamine (10.19 mL, 79.5 mmol). Then diacid chloride **169** (0.46 g, 1.62 mmol) in chloroform (5 mL) was added to the mixture dropwise at 0 °C. The reaction was stirred at 20 °C for 17 h. The mixture was evaporated then diluted with ethyl acetate (30 mL) and washed with water (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (1:1, ethyl acetate: 40-60 °C petroleum ether) to give **165** (0.22 g, 30%) as a white foam; $[\alpha_D]^{23}$= - 23.1 ° (*c*=1.0, CH_2Cl_2); IR (thin film) (ν_{max}) 3350, 2265, 1724, 1687, 1536, 1229, 1109 cm^{-1}; Rf 0.2 (EtOAc); ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.30 (2H, m, NH), 8.33 (major rotamer), 8.23 (min rotamer), (2H, s, 3,5-pyridyl), 5.18 (2H, t, *J*= 6 Hz, CH_2OH), 4.60 (2H, m, $CHCH_2OH$), 3.89 (4H, m, CH_2OH), 3.66 (6H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz); δ_c 170.39 (O-*C*=O), 162.20 (N-*C*=O), 150.13 (s, pyridyl), 135.22 (s, pyridyl, C-Br), 127.76 (d, pyridyl), 60.46 (CH_2OH), 55.39 ($CHCH_2OH$), 52.02 (OCH_3). LRMS m/z (EI) 448 ($M+^{81}$, 2%), 446 ($M+^{79}$, 4%), 419 (2%), 382 (13%), 282 (3%), 147 (72%) 106 (15%) 92 (56%) 78 (100%): HRMS calcd for $C_{15}H_{18}BrN_3O_8$ $[M]^+$ 447.02772. Found 447.02616.

4-Bromopyridine-2, 6-bisoxazoline methyl ester 164



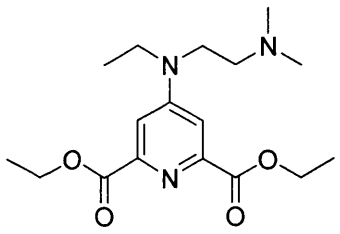
To a stirred solution of amide **165** (0.20 g, 0.45 mmol) in anhydrous dichloromethane (20 mL) at -78 °C was added DAST (0.13 g, 0.98 mmol) dropwise. The mixture was stirred at -78 °C for 4 h. Potassium carbonate (1.85 g, 0.95 mmol) was then added to the mixture, which was left to stir from -78 °C to ambient temperature over 17 h. The mixture was then poured into saturated aqueous sodium hydrogen carbonate (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give **164** (0.20 g, 89%) as a yellow oil; $[\alpha_D]^{23}$ = - 16.2 ° (*c*=1.0, CH_2Cl_2); IR (thin film) (ν_{max}) 3236, 1733, 1627, 1560, 1386, 1218, 748 cm^{-1}; Rf 0.2 (1:1 EtOAc: petroleum ether); ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.43 (2H, s, 3,5-pyridyl), 4.82 (2H, m, C*H*CH_2), 4.70 (4H, m, CHC*H*$_2$), 3.81 (6H, s, OC*H*$_3$); ^{13}C NMR ($CDCl_3$ 75 MHz) δ_c 170.79 (O-*C*=O), 163.95 (s, pyridyl), 147.36 (s, *C*=N oxazolinyl), 134.06 (s, pyridyl, *C*-Br), 129.85 (d, pyridyl), 70.64 (N-*C*HCH$_2$, oxazolinyl), 68.64 (*C*H$_2$CH, oxazolinyl), 52.89 (OCH_3). LRMS m/z +(CI) 411 (M^{81}+H, 65%), 409 (M^{79}+H, 67%), 365 (37%), 351 (8%), 331 (100%), 306 (4%) 272 (12%) 115 (3%): HRMS calcd for $C_{15}H_{14}BrN_3O_6$ $[M]^+$ 412.0144. Found 412.0139.

4-Bromopyridine-2, 6-bisoxazolyl methyl ester 145



To a stirred solution of oxazoline **164** (0.20 g, 0.48 mmol) in dichloromethane (15 mL) was added DBU (0.30 mL, 2.04 mmol). After 10 min bromotrichloromethane (0.16 mL, 1.1 mmol) was added dropwise at -10 °C. The mixture was stirred for 17 h at 20 °C. The mixture was evaporated and purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give **145** (0.10 g, 51 %) as a beige foam. IR (thin film) (ν_{max}) 3236, 1733, 1627, 1560, 1386, 1218, 748 cm^{-1}; Rf 0.2 (1:1 EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.66 (1H, s, oxazolyl), 8.44 (2H, s, pyridyl), 3.98 (6H, s, OCH_3); ^{13}C NMR ($CDCl_3$ 75 MHz) δ_c 161.11 (O-*C*=O), 158.36 (s, pyridyl), 146.47 (s, *C*=N, oxazolyl), 145.60 (d, oxazolyl, N-C=*C*H), 134.91 (s, pyridyl, *C*-Br), 127.46 (s, oxazolyl, N-*C*=CH), 127.46 (d, pyridyl), 52.51 (OCH_3); LRMS m/z (EI) 409 (M^{81}+, 1%), 407 (M^{79}+, 2%), 363 (2%), 206 (4%), 147 (100%), 119 (22%) 92 (65%) 78 (41%) 65 (6%): HRMS calcd for $C_{15}H_{10}BrN_3O_6$ $[M]^+$ 406.9753. Found 406.9749.

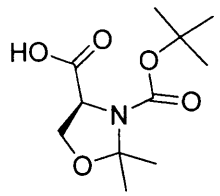
Diethyl 4-((2-(dimethyl amino) ethyl)(ethyl) amino) pyridine-2,6-dicarboxylate 171



To a suspension of 4-bromopyridine-2,6-dicarboxylate **167** (0.20 g, 0.66 mmol) in DMF (6 mL) was added anhydrous potassium carbonate (0.10 g, 0.78 mmol) and N^1-ethyl-N^2, N^2–dimethylethane-1,2-diamine (0.8 mL). The yellowish-brown mixture was heated at 60 °C for 48 h. The resultant brown suspension was cooled to room temperature and poured onto water (100 mL) and the mixture was extracted with ethyl acetate (100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The brown residue was purified via column chromatography (5% methanol: ethyl acetate) to give **171** (0.08 g 40%) as a yellow oil. IR (thin film) (ν_{max}) 3404, 1645, 1450, 1124, 1022 cm^{-1}; Rf 0.2 (EtOAc); ^1H NMR ($CDCl_3$, 300 MHz): δ_H 7.56 (2H, s, pyridyl), 4.41 (4H, m, CH_3CH_2,), 3.48 (4H, m, 2xNCH_2), 2.51 (2H, t, $CH_2N(CH_3)_2$, J = 6 Hz), 2.32 (6H, s, $N(CH_3)_2$), 1.43 (6H, t, CH_2CH_3 J = 6 Hz), 1.20 (3H, t, J = 6 Hz, NCH_2CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_c 165.86 (C=O), 153.96 (s, pyridyl), 149.13 (s, pyridyl), 109.91 (d, pyridyl), 62.14 (2xCH_2CH_3), 56.00 ($CH_2CH_2N(CH_3)_2$), 47.98 ($CH_2CH_2N(CH_3)_2$), 45.71 ($N(CH_3)_2$), 45.08 (NCH_2CH_3), 14.20 (NCH_2CH_3), 11.96 (2xCH_2CH_3); LRMS m/z +(CI) 338 (M+H, 6%), 310 (1%). 270 (30%), 197 (16%), 114 (15%), 74 (100%): HRMS calcd for $C_{17}H_{27}N_3O_4$ [M+H] 338.2074. Found 338.2084.

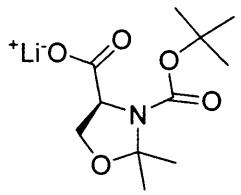
mixture was stirred for 1 h at ambient temperature. The mixture was evaporated and the residue was dissolved in dichloromethane (50 mL) and washed with aqueous solution of sodium hydrogen carbonate (50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (9:1, petroleum ether:ethyl acetate) to give **189** (5.52 g, 82 %) as a colourless oil; Rf 0.3 (1:1 EtOAc: petroleum ether); ^{1}H NMR (DMSO-d_6, 75 °C, 400 MHz) δ 4.40 (1H, m, C*H*CH_2), 4.15 (1H, dd, *J*= 8, 12 Hz, C*H*H, oxazolidinyl), 3.93 (1H, dd, *J*= 4, 8 Hz, CH*H* oxazolidinyl), 3.01 (3H, s, OCH_3), 1.55 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.40 (9H, s, CH_3, Boc); ^{13}C NMR (DMSO-d_6, 75 °C, 100 MHz) δ 170.54 (H_3C-*C*=O), 150.40 ($[CH_3]_3O$-*C*=O), 93.50 ($NC(Me)_2O$), 79.13 ($[CH_3]_3C$), 65.14 (OCH_2CH, oxazolidinyl), 58.36 (OCH_2CH, oxazolidinyl), 51.24 (OCH_3), 27.41 ($[CH_3]_3C$), 24.68 (CH_3), 23.95 (CH_3).

2,2-Dimethyloxazolidine-3,4-dicarboxylic acid 3-*tert*-butyl ester (188)[14-16]



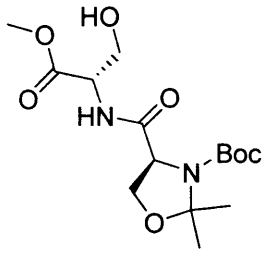
To a stirred solution of ester **189** (0.50 g, 2.04 mmol) in THF: water (20:10 mL) was added lithium hydroxide (8.5 mg, 2.04 mmol) at 20 °C. The mixture was heated at 50 °C for 14 h. The mixture was then evaporated and neutralised to pH 7. Extraction with ethyl acetate (15 mL) gave an organic layer which was dried over $MgSO_4$, filtered and evaporated to give **188** (0.31 g, 65%) as a white foam. Rf baseline (EtOAc); ^{1}H NMR (DMSO-d_6, 75 °C, 400 MHz) δ 4.27 (1H, m, C*H*CH_2, oxazolidinyl) , 4.11 (1H, m, C*H*H, oxazolidinyl), 3.93 (1H, dd, *J*=4, 8 Hz, CH*H*, oxazolidinyl), 1.55 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.40 (9H, s, CH_3, Boc); ^{13}C NMR (DMSO-d_6, 23 °C, 75 MHz) δ 174.65 (HO-*C*=O), 151.63 ($[CH_3]_3O$-*C*=O), 92.94 ($NC(Me)_2O$), 78.42 ($[CH_3]_3C$), 67.16 (OCH_2CH, oxazolidinyl), 61.01 (OCH_2CH, oxazolidinyl), 28.09 ($C[CH_3]_3$), 25.31 (CH_3), 24.48 (CH_3).

2,2-Dimethyl-oxazolidine3,4-dicarboxylic acid 3-*tert*-butyl ester 191



To a stirred solution of ester **189** (0.50 g, 2.04 mmol) in THF: water (20:10 mL) was added lithium hydroxide (8.5 mg, 2.04 mmol) at 20 °C. The mixture was heated to 50 °C for 14 h. The mixture was evaporated and ethyl acetate (15 mL) was added. The organic layer (containing organic impurities) was discarded. The aqueous layer was evaporated afford **191** (0.55 g, 100%) as a white solid, mp 222 °C (dec).

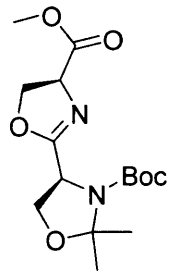
4-(2-Hydroxy-1-methoxycarbonyl-ethylcarbammyl)2,2-dimethoxy-oxazolidine-3-carboxylic acid-*tert*-butyl ester (187)[14,15]



To a stirred solution of lithium carboxylate **191** (0.50 g, 2.04 mmol) in anhydrous THF (20 mL) was added triethylamine (0.55 mL, 4.32 mmol). Then isobutyl chloroformate (0.28 mL, 2.16 mmol) was added to the mixture at -30 °C and stirred for 3 h. Then L-serine methyl ester hydrochloride (0.67 g, 4.32 mmol) was added in one portion. The thick slurry was stirred at ambient temperature for 16 h. The mixture was evaporated and the residue was extracted with ethyl acetate (20 mL) and water (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to give **187** (0.70 g, 100%) as a colourless oil; Rf 0.2 (EtOAc); ^{1}H NMR (DMSO-d_6, 90 °C, 400 MHz) δ_H 7.80 (1H, bs, NH), 4.62 (1H, m, C*H*CH_2, oxazolidinyl), 4.39 (2H, m, C*H*$_2$OH), 4.08 (1H, m, C*H*CH_2OH), 3.85 (1H, m, CH*H*, oxazolidinyl), 3.78 (1H, m, CH*H*, oxazolidinyl), 3.65 (3H, s, OCH_3) 1.55 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.38 (9H, s, $[CH_3]_3$ Boc); ^{13}C NMR

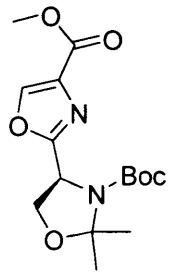
(DMSO-d_6, 75 °C, 100 MHz) δ_C 170.69 (HN-*C*=O), 170.22 (H_3CO-*C*=O), 151.20 ($[CH_3]_3O$-*C*=O), 93.86 (N*C*$(Me)_2O$), 79.42 ($[CH_3]_3C$), 66.29 (OCH_2CH, oxazolidinyl), 61.30 (CH_2OH), 59.47 (OCH_2CH, oxazolidinyl), 54.58 ($CHCH_2OH$), 51.62 (OCH_3), 27.92 ($[CH_3]_3C$), 25.32 (CH_3), 24.37 (CH_3): HRMS calcd for $C_{15}H_{26}N_2O_7$ [M] 346.1740. Found 346.1720.

4-(Methoxycarbonyl) oxazoline-2,2 dimethoxy-oxazolidine-3-carboxylic acid-*tert*-butyl ester (186)[15]



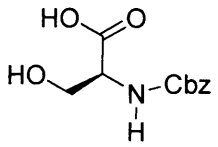
To a stirred solution of amide **187** (0.34 g , 0.98 mmol) in anhydrous dichloromethane (20 mL) was added DAST (0.38 mL, 2.87 mmol) dropwise at -78 °C. The mixture was stirred at -78 °C for 4 h. Potassium carbonate (0.47 g, 3.42 mmol) was then added to the mixture, which was stirred from -78 °C to ambient temperature over 17 h. The mixture was then poured into a solution of sodium hydrogen carbonate (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give **186** (0.25 g, 77 %) as a yellow oil; IR (thin film) (ν_{max}) 3429, 2979, 1743, 1699, 1392, 1249, 1170 cm^{-1}; Rf 0.3 (EtOAc); ^1H NMR (DMSO-d_6, 75 °C, 400 MHz) δ_H 4.74 (1H, m, C*H*CH_2, oxazolinyl), 4.57 (1H, m, C*H*CH_2, oxazolidinyl), 4.41 (1H, m, CHC*H*$_2$, oxazolinyl), 4.13 (1H, dd, *J*= 6.5, 8.9 Hz, CH*H*, oxazolidinyl), 3.91 (1H, dd, *J*= 2.9, 8.9 Hz, CH*H*, oxazolidinyl), 3.68 (3H, s, OCH_3) 1.54 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.39 (9H, s, CH_3, Boc); ^{13}C NMR (DMSO-d_6, 75 °C, 100 MHz) δ_C 171.05 (O-C=O), 167.99 ($[CH_3]_3O$-*C*=O), 155.42 (s, oxazolinyl), 93.86 (N*C*$(Me)_2O$), 79.29 ($[CH_3]_3C$), 69.75 (CH_2CH, oxazolinyl), 67.72 (OCH_2CH, oxazolidinyl), 66.23 (CH_2CH, oxazolinyl), 54.10 (OCH_2CH, oxazolidinyl), 52.05 (OCH_3), 27.73 ($[CH_3]_3C$), 24.84 (CH_3), 23.89 (CH_3): HRMS calcd for $C_{15}H_{24}N_2O_6$ [M+Na] 351.1532. Found 351.1528.

4-(Methoxycarbonyl) oxazolyl-2,2 dimethoxy-oxazolidine-3-carboxylic acid-*tert*-butyl ester (185)[14,15]



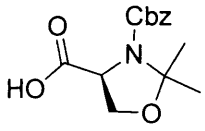
To a stirred solution of oxazoline **186** (0.20 g, 0.61 mmol) in anhydrous dichloromethane (10 mL) was added DBU (0.18 g, 1.22 mmol) and stirred at ambient temperature for 10 min. Then bromotrichloromethane (0.07 mL, 0.67 mmol) was added to the mixture at -10 °C dropwise. The mixture was allowed to warm up with stirring from -10°C to ambient temperature over 20 h. The mixture was evaporated and the residue was purified by column chromatography (1:3 ethyl acetate: petroleum spirit) to give **185** (0.18 g, 89%) as a white solid mp 125-127 °C, lit.[15] mp 124 °C ; Rf 0.35 (EtOAc); ^{1}H NMR (DMSO-d_6, 75 °C, 400 MHz) δ_H 8.66 (1H, s, oxazolyl), 5.08 (1H, m, C*H*CH_2, oxazolidinyl), 4.26 (1H, dd, *J*= 6.60, 9.2 Hz, CH*H*, oxazolidinyl), 4.02 (1H, dd, CH_2, *J*= 3.1, 9.2 Hz, CH*H*, oxazolidinyl), 3.81 (3H, s, OCH_3) 1.63 (3H, s, CH_3), 1.52 (3H, s, CH_3), 1.32 (9H, s, CH_3, Boc); ^{13}C NMR (DMSO-d_6, 75 °C, 100 MHz) δ_C 163.29 (H_3CO-*C*=O), 160.49 ($[CH_3]_3O$-*C*=O), 150.43 (s, O-C=N oxazolidinyl), 144.50 (s, *C*CHO, oxazolyl), 132.23 (d, CH, oxazolyl), 93.71 (N*C*$(Me)_2O$), 79.42 ($[CH_3]_3$*C*), 66.30 (O*C*H_2CH, oxazolidinyl), 54.08 (OCH_2*C*H, oxazolidinyl), 51.05 (OCH_3), 27.38 ($[CH_3]_3$C), 25.10 (CH_3), 23.79 (CH_3).

2-Benzyloxycarbonylamino-3-hydroxy-propionic acid (202)[17]



L-Serine (10.0 g, 95 mmol) was dissolved in 2 M NaOH: dioxane (80:40 mL). The temperature of the reaction was lowered to 0 °C and then benzyl chloroformate (15 mL, 104 mmol) was added dropwise. The mixture was stirred to ambient temperature for 48 h. The mixture was then acidified to pH 4 using 3M HCl and extracted with ethyl acetate (150 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (1% ethyl acetate: petroleum ether) to give **202** (13.0 g, 57 %) as a white solid, mp 119-120 °C, lit.[17] mp 120-122 °C; Rf 0.2 (1:2 EtOAc: petroleum ether); ^{1}H NMR (DMSO-d_6, 300 MHz): δ_H 7.35 (5H, m, aryl), 5.03 (2H, s, CH_2Ph), 4.07 (1H, m, CHCH$_2$OH), 3.73 (2H, m, CH_2OH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_c 172.06 (HO-C=O), 155.97 (PhCH$_2$O-C=O), 136.90 (s, aryl), 128.28 (d, aryl), 127.74 (d, aryl), 127.66 (d, aryl), 65.39 (CH$_2$Ph), 61.26 (CHCH$_2$OH), 56.58 (CHCH$_2$OH).

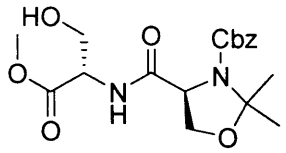
2, 2-Dimethyl-oxazolidine-3, 4-dicarboxylic acid 3-benzyl ester (201)[14,18]



To a stirred solution of acid **202** (5.25 g, 22 mmol) in acetone (100 mL) was added 2, 2-dimethoxypropane (24 mL, 270 mmol). *p*-Toluenesulfonic acid (0.58 g, 3.0 mmol) in acetone (30 mL) was added to the mixture dropwise. The mixture was stirred for 12 h at 45 °C, then evaporated and basified to pH 8 using saturated sodium hydrogen carbonate. After extraction with ethyl acetate (60 mL), the aqueous layer was acidified to pH 1 using 3M HCl. Ethyl acetate (150 mL) was added and the organic layer was dried over $MgSO_4$, filtered and

evaporated to give **201** (6.13 g, 100%) as a white solid, mp 110 °C. Rf 0.2 (1:1 EtOAc: petroleum ether); ^{1}H NMR (DMSO-d_6, 90°C, 400 MHz): δ_H 7.35 (5H, m, aryl), 5.06 (2H, m, C*H*$_2$Ph), 4.44 (1H, m, C*H*CH$_2$, oxazolidinyl), 4.19 (1H, dd, *J*= 7.2, 9.1 Hz, CH*H* oxazolidinyl), 4.05 (1H, dd, *J*= 2.5, 9.1 Hz, CH*H* oxazolidinyl), 1.58 (3H, s, CH_3), 1.48 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90 °C, 400 MHz) δ_c 172.03 (OH-C=O), 152.03 (PhCH$_2$O-*C*=O), 136.57 (s, aryl), 128.28 (d, aryl), 127.64 (d, aryl), 126.94 (d, aryl), 94.22 (N*C*(Me)$_2$O), 66.24 (O*C*H$_2$Ph, oxazolidinyl), 65.74 (O*C*H$_2$CH, oxazolidinyl), 58.25 (OCH$_2$*C*H, oxazolidinyl), 24.88 (CH_3), 23.62 (CH_3).

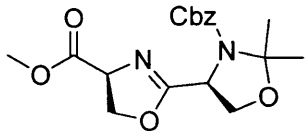
4-(2-Hydroxy-1-methoxycarbonyl-ethyl carbamoyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid benzyl ester (200)[14]



To a stirred solution of acid **201** (3.07 g, 10.0 mmol) in anhydrous dichloromethane (30 mL) triethylamine (2.79 mL, 22 mmol) was added. The mixture was left to stir for 10 min and isobutyl chloroformate (1.59 mL, 12 mmol) was added dropwise at -30 °C. The mixture was stirred for 2.5 h at -30 °C and L-serine methyl ester (2.54 g, 16.0 mmol) was added. The mixture was stirred for 17 h at ambient temperature. Water (30 mL) was added to the reaction the organic layer was dried over $MgSO_4$, filtered and evaporated to give **200** (3.55 g, 85%) as a colourless oil; Rf 0.2 (EtOAc); ^{1}H NMR (DMSO-d_6, 90 ° C, 400 MHz) δ_H 7.91 (1H, d, *J*=8 Hz, N-H), 7.33 (5H, m, aryl), 5.06 (2H, s, C*H*$_2$Ph), 4.75 (1H, t, *J*= 5.4 Hz, C*H*CH$_2$, oxazolidinyl), 4.52 (1H, m, C*H*CH$_2$OH), 4.39 (2H, m, CHC*H*$_2$OH), 4.16 (1H, dd, *J*= 7.1, 8.9 Hz, C*H*H oxazolidinyl), 3.93 (1H, dd, *J*= 3.2, 9.0 Hz, CH*H* oxazolidinyl), 3.72 (3H, m, OCH_3), 1.64 (3H, s, CH_3), 1.58 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6 90 ° C, 400 MHz): δ_c 171.24 (HN-*C*=O), 170.50 (H_3CO-*C*=O), 152.27 (PhCH$_2$O-*C*=O), 137.16 (s, aryl), 128.63 (d, aryl), 128.01 (d, aryl), 127.65 (d, aryl), 94.90 (N*C*(Me)$_2$O), 67.30 (*C*H$_2$Ph), 66.55 (O*C*H$_2$CH, oxazolidinyl), 61.83 (O*C*HCH$_2$,

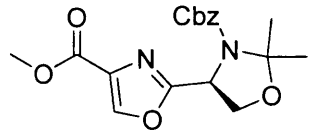
oxazolidinyl), 59.83 (CH_2OH), 55.31 ($CHCH_2OH$), 52.24 (OCH_3), 25.61 (CH_3), 24.91 (CH_3).

4-(2-Hydroxy-1-methoxycarbonylethyl carbamoyl)-2,2-dimethyloxazolidine-oxazoline methyl ester 199



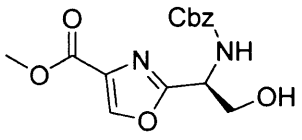
To a stirred solution of amide **200** (4.27 g, 11 mmol) in anhydrous dichloromethane (30 mL) was added DAST (1.78 mL, 13 mmol) dropwise at -78°C. The mixture was left to stir at -78 °C for 2 h. Potassium carbonate (2.80 g, 21.0 mmol) was then added to the mixture, which was stirred at ambient temperature for 16 h. The mixture was then poured into a solution of sodium carbonate (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (3:1 hexanes: ethyl acetate) to give **199** (2.97 g, 73 %) as a colourless oil; $[\alpha_D]^{23}$= -11° (*c*=1.0, CH_2Cl_2); IR (thin film) (ν_{max}) 2985, 1712, 1411, 1350, 1211, 1095, 841 cm^{-1}; Rf 0.2 (1:3EtOAc: petroleum ether); ^1H NMR (DMSO-d_6, 90°C, 400 MHz): δ_H 7.36 (5H, m, aryl), 5.14 (1H, m, C*H*CH_2, oxazolinyl), 5.10 (1H, m, C*H*CH_2, oxazolidinyl), 4.90 (1H, d, J_{HA}=12.7 Hz, C*H*HPh), 4.83 (1H, d, J_{HB}=12.7 Hz, CH*H*Ph), 4.40 (2H, m, CHCH_2, oxazolinyl), 4.21 (1H, dd, *J*= 2.0, 8.7 Hz, CH*H*, oxazolidinyl), 4.03 (1H, dd, *J*= 2.0, 8.7 Hz, CH*H*, oxazolidinyl), 3.71 (3H, s, OCH_3), 1.63 (3H, s, CH_3), 1.52 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90°C, 100 MHz) δ_c 171.20 (H_3CO-C=O), 168.18 ($PhCH_2O$-C=O), 151.89 (s, C=N), 137.01 (s, aryl), 128.60 (d, aryl), 128.02 (d, aryl), 127.68 (d, aryl), 94.82 (N*C*$(Me)_2O$), 70.37 (O*C*H_2CH, oxazolinyl), 68.23 (OCH_2*C*H, oxazolinyl), 66.94 (O*C*H_2Ph), 66.49 (O*C*H_2CH, oxazolidinyl), 54.67 (OCH_2*C*H, oxazolidinyl), 52.28 (OCH_3), 25.60 (CH_3), 24.56 (CH_3); LRMS m/z (EI) 338 (M^+, 8%), 347 (38%). 303 (64%), 213 (2%), 156 (4%), 123 (1%): HRMS calcd for $C_{18}H_{22}N_2O_6$ $[M]^+$ 362.1478. Found 362.1466.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-oxazole methyl ester (198)[14]



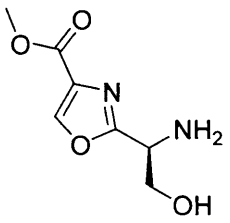
To a stirred solution of oxazoline **199** (4.13 g, 11 mmol) in anhydrous dichloromethane (30 mL) was added DBU (3.4 mL, 22.0 mmol). Then bromotrichloromethane (1.24 mL, 12.5 mmol) was added to the mixture at -10 °C dropwise. The mixture was allowed to warm up with stirring from -10°C to ambient temperature over 20 h. The mixture was evaporated and the residue was purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give **198** (3.08 g, 75%) as a colourless oil; Rf 0.2 (1:1 EtOAc: petroleum ether); ^{1}H NMR (DMSO-d_6, 90°C, 400 MHz) δ_H 8.60 (1H, s, oxazolyl), 7.28 (5H, m, aryl), 5.21 (1H, m, C*H*CH_2, oxazolinyl), 5.10 (1H, d, J_{HA}=12.7, C*H*HPh), 4.99 (1H, d, J_{HB}=12.8, CH*H*Ph), 4.30 (1H, dd, J= 6.5, 9.3 Hz, C*H*H, oxazolidinyl), 4.07 (1H, dd, J= 2.6, 9.3 Hz, C*H*H oxazolidinyl), 3.81 (3H, s, OCH_3), 1.64 (3H, s, CH_3), 1.53 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90°C, 100 MHz) δ_c 162.98 (H_3CO-*C*=O), 160.61 ($PhCH_2O$-*C*=O), 151.67 (s, *C*=N, oxazolyl), 144.85 (d, C=*C*H, oxazolyl), 135.93 (s, *C*=CH, oxazolyl), 132.31 (s, aryl), 127.87 (d, aryl), 127.40 (d, aryl), 126.96 (d, aryl), 94.31 (N*C*$(Me)_2O$), 66.70 (O*C*H_2Ph), 66.00 (O*C*H_2CH, oxazolidinyl), 54.12 (OCH_2*C*H, oxazolidinyl), 51.24 (OCH_3), 25.19 (CH_3), 23.68 (CH_3); HRMS calcd for $C_{18}H_{20}N_2O_6$ [M+Na] 383.1219. Found 383.1212.

2-(1-Benzyloxycarbonylamino-2-hydroxy-ethyl) oxazole-4-carboxylic acid methyl ester (204)



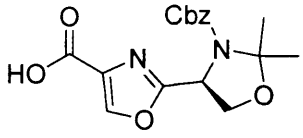
To a stirred solution of oxazolidine **198** (0.82 g, 2.24 mmol) in methanol (30 mL) was added *p*-toluenesulfonic acid (0.43 g, 2.24 mmol). The reaction was heated at reflux for 2.5 h. The mixture was then evaporated and purified by column chromatography (1:4 ethyl acetate: 40-60 °C petroleum ether) to give **204** (0.73 g, quantitative) as a white solid, mp 101-102 °C; $[\alpha_D]^{23}$= -14 ° (*c*=1.0, CH_2Cl_2); IR (thin film) (ν_{max}) 3402, 1720, 1585, 1259, 1111, 1028, 912 cm^{-1}; Rf 0.2 (1:1 EtOAc: petroleum ether); ^1H NMR (DMSO-d_6, 90 °C, 400 MHz) δ_H 8.43 (1H, s, oxazolyl), 7.24 (1H, m, NH), 7.12 (5H, m, aryl), 4.89 (2H, m, CH_2Ph), 4.61 (1H, m, C*H*CH_2OH), 3.60 (2H, m, CH_2OH), 3.57 (1H, m, OCH_3); ^{13}C NMR (DMSO-d_6, 90 °C, 100 MHz) δ_c 163.93 (CH_3OC=O), 161.44 (CH_2OC=O), 156.09 (s, oxazolyl), 145.44 (s, aryl), 137.26 (d, oxazolyl), 132.88 (s, oxazolyl), 128.61 (d, aryl), 128.06 (d, aryl), 127.90 (d, aryl), 66.18 (CH_2OH), 62.30 (CH_2OC=O), 52.31 (C*H*CH_2OH), 51.87 (OCH_3); LRMS m/z (EI) 320 (M^+, 26%), 290 (13%), 245 (6%), 213 (3%), 199 (22%), 91 (100%): HRMS calcd for $C_{15}H_{16}N_2O_6$ [M] 320.1008. Found 320.1009. *Anal.* Calcd for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04; N, 8.75. Found: C, 55.85; H, 5.18; N, 8.47.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2,2-dimethyloxazolidine-tris oxazolyl carboxylic acid (105)[14]



To an evacuated solution of carbamate **204** (2.23 g, 6.96 mmol) in methanol (50 mL) was added 10% palladium-on-carbon (0.223 g, 0.69 mmol). The mixture was evacuated and hydrogen was admitted. The mixture was stirred for 17 h, then evacuated and finally air admitted. The mixture was filtered through a pad of celite and the filtrate was evaporated to give **105** (1.2 g, 92 %) as a brown oil; Rf baseline (EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.25 (1H, s, oxazolyl), 4.32 (1H, m, C*H*CH_2), 4.02 (2H, m, CH_2OH), 3.91 (1H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_c 162.54 (H_3CO-*C*=O), 161.52 (s, *C*=N, oxazolyl), 144.40 (d, C=*C*H, oxazolyl), 133.04 (s, *C*=CH, oxazolyl), 63.87 (*C*H_2OH), 52.29 (*C*HCH_2OH), 51.42 (O*C*H_3): HRMS calcd for $C_7H_{10}N_2O_4$ [M+Na] 209.0538. Found 209.0531.

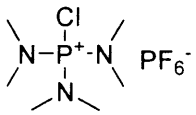
4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-oxazole carboxylic acid (197)[14]



To a stirred solution of ester **198** (0.60 g, 1.66 mmol) in THF: water (8:1 mL) was added lithium hydroxide (0.08 g, 1.99 mmol) in water (2 mL). The mixture was stirred for 17 h at reflux. The mixture was evaporated and the residue was acidified to pH 1. Ethyl acetate (20 mL) was added to the mixture was extracted with water (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **197** (0.46 g, 81 %) as white platelets, mp 130-133 °C; Rf 0.2 (EtOAc); (DMSO, 90 °C, 400 MHz) ^{1}H NMR δ_H 8.50 (1H, s, oxazolyl), 7.21

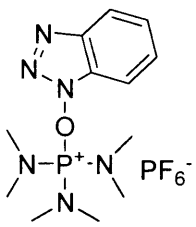
(5H, m, aryl), 5.20 (1H, m, C*H*CH_2, oxazolidinyl), 5.11 (1H, d, J_{HA}= 12.4 Hz, C*H*HPh), 5.01 (1H, d, J_{HB}= 12.8 Hz, CH*H*Ph), 4.28 (1H, dd, *J*= 6.5, 9.3 Hz, C*H*H, oxazolidinyl), 4.16 (1H, dd, *J*= 2.6, 9.3 Hz, CH*H* oxazolidinyl), 1.65 (1H, s, CH_3), 1.47 (1H, s, CH_3); ^{13}C NMR (DMSO, 90°C, 100 MHz) δ_c 163.42 (HO-*C*=O), 162.03 ($PhCH_2O$-C=O), 151.91 (s, C=N, oxazolyl), 145.10 (d, C=*C*H, oxazolyl), 136.70 (s, *C*=CH, oxazolyl), 134.12 (s, aryl), 128.57 (d, aryl), 128.07 (d, aryl), 127.63 (d, aryl), 95.00 (N*C*$(Me)_2O$), 67.46 (OCH_2Ph), 66.68 (OCH_2CH, oxazolidinyl), 54.89 (OCH_3), 25.92 (CH_3), 24.42 (CH_3): HRMS calcd for $C_{17}H_{18}N_2O_6$ [M+H] 347.1243. Found 347.1238.

Chlorotris(dimethylamino)phosphonium hexafluorophosphate(V) (241)[19]



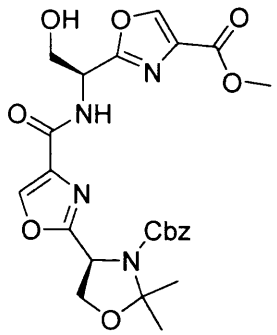
Potassium hexafluorophosphonate (9.25 g, 50 mmol) was dispersed in acetonitrile (250 mL). Oxalyl chloride (4.3 mL, 50 mmol) and DMF (2.5 mL, 30 mmol) were added under inert atmosphere. To the mixture was added HMPA (8.75 mL, 50 mmol) at 0 °C dropwise. The mixture was left to stir for 4 h at 20 °C. The mixture was filtered and the filtrate was evaporated to give **241** (10.9 g, 64 %) as a white solid, mp 109 °C.

(1*H*-benzo[*d*][1,2,3]triazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (242)[20]



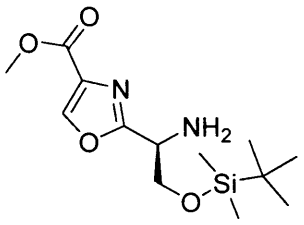
To a suspension of phosphate **241** (10.9 g, 31.8 mmol) in acetone (150 mL) HOBT (4.30 g, 31.8 mmol) and triethylamine (4.07 mL, 31.8 mmol) was added. The mixture was stirred for 1 h and then filtered. The filtrate was evaporated to give **242** (11 g, 78%) as a white solid mp 137-138 °C.

2-(1-Amino-2-hydroxy-ethyl)-oxazole-4-carboxylic acid methyl ester (Hydroxy-1-methoxycarbonyl ethyl carbomyl)-2, 2-dimethyloxazolidine-oxazole (196)[14]



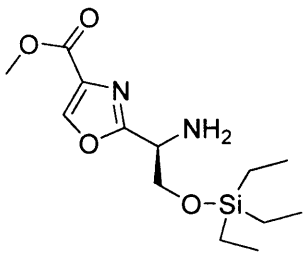
To a solution of acid **197** (1.0 g, 2.87 mmol) in DMF (250 mL) was added BOP (1.5 g. 3.4 mmol), DIEA (1.2 mL, 6.60 mmol) and HOBT (0.43 g, 3.51 mmol). The mixture was stirred for 10 min at -62 °C. Then a solution of amine **105** (0.45 g, 2.41 mmol) in DMF (20 mL), pre-cooled at -62 °C, was added to the mixture, dropwise via a cannula. The mixture was left to stir for 72 h at -62 °C; then ethyl acetate (150 mL) was added and this mixture was extracted with water (3x 150 mL). The aqueous layer was extracted with ethyl acetate (3x 150 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **196** (0.10 g, 77 %) as a colourless oil used in the next step without purification. Rf 0.2 (1:2 EtOAc: petroleum ether); (DMSO-d_6, 80 °C, 400 MHz) ^{1} H NMR δ_H 8.67 (1H, s, oxazolyl), 8.47 (1H, s, oxazolyl), 8.09 (1H, d, *J*=8 Hz, N*H*), 7.25 (5H, m, aryl), 5.22 (2H, m, CH_2OH), 5.20 (1H, m, C*H*CH_2OH), 5.03 (2H, m, CH_2O-C=O), 4.31 (1H, dd, *J*=6.8, 9.6 Hz, OC*H*H oxazolidinyl), 4.12 (1H, dd, *J*=6.4, 9.6 Hz, OCH*H* oxazolidinyl), 4.10 (1H, t, *J* =5.2 Hz, O*H*), 3.82 (3H, s, OCH_3), 1.67 (3H, s, CH_3), 1.55 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90 °C, 100 MHz); δ_c 163.00 (CH_3O-C=O), 162.69 (C=O, Cbz), 160.99 (HN-C=O), 159.65 (s), 145.12 (s), 145.09 (s), 142.11 (s), 136.22 (d), 135.63 (d), 132.50 (s), 128.191 (d), 127.69 (d), 127.24 (d), 94.64 (N*C*(Me_2)O), 67.03 (*C*H_2OCO, Cbz), 66.35 (OCH_2CH, oxazolidine), 61.72 (OCH_2*C*H, oxazolidine), 54.50 (OCH_3), 51.50 (*C*H_2OH), 49.50 (N*C*HCH_2O), 25.57 (CH_3), 24.01 (CH_3): HRMS calcd for $C_{30}H_{28}N_6O_{11}$ [M+H] 515.1778. Found 515.1801.

Methyl 2-(1-amino-2-(*tert*-butyldimethylsilyloxy) ethyl) oxazole-4-carboxylate (205)



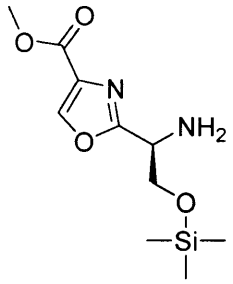
Imidazole (0.18 g, 2.68 mmol), TBSCl (2.5 mL, 1.35 mmol) and DMAP (0.70 mg, 0.054 mmol) were added sequentially to a suspension of amine **105** (0.1 g, 0.54 mmol) in dichloromethane (5' mL) at ambient temperature. Then the mixture was washed with water (2 mL), the organic layer was dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give **205** (0.074 g, 46%) as a yellow oil; $[\alpha_D]^{23}$= -23 °(*c*=0.8, $CHCl_3$); IR (thin film) (ν_{max}) 3299, 2953, 1745, 1664, 1585, 1112, 1002 cm^{-1}; Rf 0.2 (EtOAc); ^1H NMR ($CDCl_3$, 300 MHz): δ_H 8.20 (1H, s, oxazolyl), 6.65 (1H, d, *J*= 6 Hz, N*H*$_2$), 5.36 (1H, m, H_2NCH), 4.02 (2H, m, O*CH*$_2$), 3.95 (1H, s, OCH_3), 0.78 (15H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_c 162.81 (C=O), 160.59 (s, oxazolyl), 144.27 (s, oxazolyl), 133.39 (d, oxazolyl), 63.98 (*CH*$_2$OH), 52.27 (NC*H*CH$_2$), 48.27 (OCH_3), 25.61 (CH_3), 18.08 (Si-CH_3); LRMS m/z +(CI) 329 (100%), 301 (M+H, 45%), 267 (2%), 197 (74%), 165 (4%), 133 (6%), 97 (44%), 85 (41%): HRMS calcd for $C_{13}H_{24}N_2O_4Si$ $[M+H]^+$ 301.1578. Found 301.1572.

Methyl 2-(1-amino-2-(triethylsilyloxy) ethyl) oxazole-4-carboxylate (206)



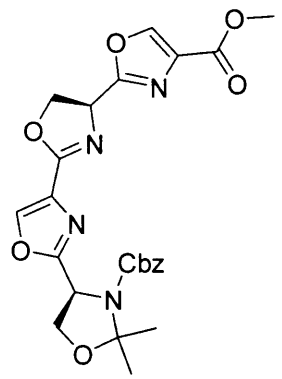
To a suspension of amine **105** (0.10 g, 0.54 mmol) in dichloromethane (5 mL) was added imidazole (0.18 g, 2.68 mmol), TESCl (2.3 mL, 1.35 mmol) and DMAP (0.7 mg, 0.054 mmol). Then the mixture was washed with water (2 mL) and the organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (ethyl acetate) to give **206** (0.08 g, 50 %) as a yellow oil; $[\alpha_D]^{23}$= -26 ° (*c*= 0.95, $CHCl_3$); IR (thin film) (ν_{max}) 3293, 2924, 1736, 1664 cm^{-1}; Rf 0.2 (EtOAc); H^1 NMR ($CDCl_3$, 300 MHz): δ_H 8.19 (1H, s, oxazolyl), 6.73 (1H, d, *J*=9 Hz, NH_2), 5.37 (1H, m, CH), 4.13 (2H, m, OCH_2), 3.95 (1H, s, OCH_3) 0.91 (6H, m, $SiCH_2$), 0.53 (9H, t, *J*=15 Hz, $SiCH_2CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_c 162.83 (C=O), 160.67 (s), 144.30 (s), 133.38 (d), 63.62 (CH_2OH), 52.27 ($CHCH_2OH$), 48.23 (OCH_3), 6.49 (CH_3), 4.11 (Si-CH_2); LRMS m/z +(CI) 329 (62%), 301 (M+H, 24%), 281 (11%), 197 (100%), 115 (32%), 85 (92%); HRMS calcd for $C_{13}H_{24}N_2O_4Si$ $[M+H]^+$ 301.1578. Found 301.1568.

2-(1-Amino-2-trimethylsilanyloxy-ethyl)-oxazole-4-carboxylic acid methyl ester (207)



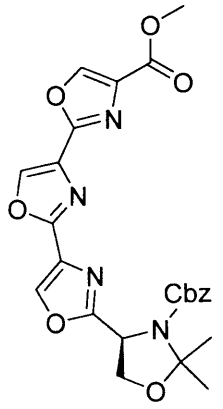
To a stirred solution of amine **105** (0.10 g, 0.53 mmol) in DMF (2 mL) was added triethylamine (0.14 mL, 1.07 mmol) and DMAP (0.013 g, 0.11 mmol). The mixture was stirred at 0 °C and trimethylsilyl chloride (0.07 mL, 0.54 mmol) was added dropwise. After stirring for 17 h at ambient temperature, ethyl acetate (10 mL) was added and the mixture was washed with water (3x10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **207** (0.08 g, 60%) as a yellow oil; $[\alpha_D]^{23}$= -25 °(*c*=0.91, $CHCl_3$); IR (thin film) (ν_{max}) 3024, 2916, 1735, 1668, 1448, 1326, 1060 cm^{-1}; Rf 0.3 (EtOAc); ^1H NMR ($CDCl_3$, 300 MHz): δ_H 8.19 (1H, s, oxazolyl), 4.01 (1H, m, C*H*CH_2Si), 3.89 (2H, s, OCH_2Si), 3.89 (1H, s, OCH_3), 0.055 (9H, s, SiCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_c 166.37 (*C*=O), 161.65 (s), 144.19 (s), 133.19 (d), 65.36 (CH_2OSi), 64.68 (*C*HCH_2), 52.20 (OCH_3), -0.66 (CH_3); LRMS m/z +(CI) 259 (M+H, 9%), 243 (3%), 197 (8%), 187 (100%), 169 (28%), 155 (94%), 128 (91%), 96 (46%): HRMS calcd for $C_{10}H_{18}N_2O_4Si$ $[M+H]^+$ 259.1114. Found 259.1116.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2,2-dimethyloxazolidine-oxazole-oxazolinyl-oxazolyl methyl ester 195



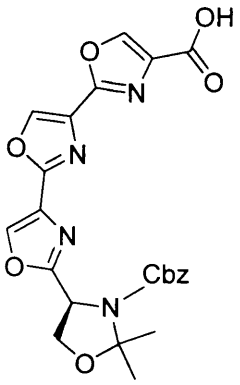
To a stirred solution of amide **196** (0.61 g, 1.19 mmol) in anhydrous dichloromethane (20 mL) at -78°C was added DAST (0.19 mL, 1.42 mmol) dropwise. The mixture was stirred for 2.5 h and then potassium carbonate (0.25 g, 1.77 mmol) was added. After 17 h water (30 mL) was added to the reaction and the mixture was extracted with dichloromethane (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **195** (0.42 g, 72 %) as an orange oil; $[\alpha_D]^{23}$= -11° (*c*=1.0, CH_2Cl_2); IR (thin film) (ν_{max}) 3136, 1714, 1643, 1406, 1348, 1095, 752 cm^{-1}; Rf 0.3 (1:1 EtOAc: petroleum ether); ^1H NMR (DMSO-d_6, 90 °C, 400 MHz): δ_H 8.68 (1H, s, oxazolyl), 8.48 (1H, s, oxazolyl), 7.28 (5H, m, aryl), 5.56 (1H, m, C*H*CH_2, oxazolinyl), 5.21 (1H, m, NC*H*CH_2, oxazolidinyl), 5.10 (1H, d, J_{HA} =12.6 Hz, C*H*HPh), 4.99 (1H, d, J_{HB} =12.6 Hz, CH*H*Ph), 4.73 (2H, m, CHCH_2, oxazolinyl), 4.31 (1H, m, C*H*H, oxazolidinyl), 4.09 (1H, m, oxazolidinyl), 3.82 (3H, s, OCH_3), 1.68 (3H, s, CH_3), 1.57 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90 °C, 100 MHz) δ_c 163.12 (PhO-*C*=O), 162.84 (H_3CO-*C*=O), 160.45 (s), 158.66 (s), 151.08 (s), 145.31 (d), 145.27 (d), 142.41 (s), 135.83 (s), 132.17 (s), 129.23 (d), 127.76 (d), 127.24 (d), 126.82 (d), 94.19 (N*C*(Me_2)O), 69.82 (t, *C*H_2CH, oxazolinyl), 66.62 (*C*H_2OCO), 65.88 (O*C*H_2CH), 65.88 (d, CH_2*C*H, oxazolinyl), 62.68 (OCH_2*C*H, oxazolidinyl), 51.42 (OCH_3), 25.10 (CH_3), 23.64 (CH_3); LRMS m/z (EI) 496 (M^+, 11%), 437 (9%), 354 (6%), 275 (25%), 215 (36%), 153 (43%), 121 (100%): HRMS calcd for $C_{24}H_{24}N_4O_8$ $[M]^+$ 496.1594. Found 496.1583.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-tris oxazolyl methyl ester (194)[14]



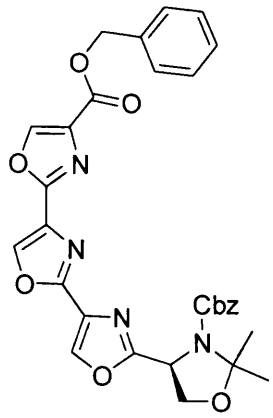
To a stirred solution of oxazoline **195** (0.38 g, 0.77 mmol) in anhydrous dichloromethane (10 mL) was added DBU (0.23 mL, 1.53 mmol). After 10 min bromotrichloromethane (0.08 mL, 0.84 mmol) was added dropwise at -10 °C. The mixture was stirred for 17 h at 20 °C. The mixture was evaporated and purified by column chromatography (1:1 ethyl acetate: petroleum ether) to give **194** (0.26 g, 69 %) as a white solid, mp 206-208 °C, lit.[14] mp 208 °C; Rf 0.5 (EtOAc); H^1 NMR (DMSO-d_6, 90°C, 400 MHz): δ_H 8.91 (1H, s, oxazolyl), 8.83 (1H, s, oxazolyl), 8.76 (1H, s, oxazolyl), 7.25 (5H, m, aryl), 5.28 (1H, m, C*H*CH_2, oxazolidinyl), 5.12 (1H, d, J_{HA} =12.6 Hz, C*H*HPh), 5.02 (1H, d, J_{HB} =12.9 Hz, CH*H*Ph), 4.33 (1H, dd, *J*=6.5, 9.2 Hz, C*H*H, oxazolidinyl), 4.15 (1H, dd, *J*=2.6, 9.3 Hz, CH*H*, oxazolidinyl), 3.86 (1H, s, OCH_3), 1.69 (3H, s, CH_3), 1.56 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90°C, 100 MHz) δ_c 163.55 (H_3CO-C=O), 160.32 ($PhCH_2C$=O), 155.17 (s), 154.51 (s), 151.06 (s), 144.74 (d), 140.42 (d), 140.22 (d), 135.84 (s), 133.09 (s), 129.65 (s), 128.74 (s), 127.70 (d), 127.21 (d), 126.84 (d), 94.22 (N*C*(Me_2)O), 66.61 (*C*H_2Ph, Cbz), 65.88 (O*C*H_2CH,oxazolidine), 52.13 (OCH_3), 51.23 (OCH_2*C*H, oxazolidine), 25.09 (CH_3), 23.61 (CH_3): HRMS calcd for $C_{24}H_{22}N_4O_8$ [M+H] 495.1515. Found 495.1506.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-tris oxazolyl carboxylic acid (193)[14]



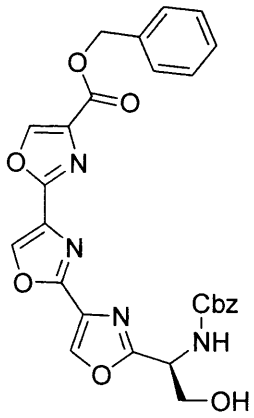
To a stirred solution of the ester **194** (0.27 g, 0.55 mmol) in THF (10 mL) was added lithium hydroxide (0.027 mL, 0.66 mmol) in water (1.5 mL). The mixture was heated at reflux for 16 h. Then the mixture was evaporated and acidified to pH 1 using 3M HCl. Ethyl acetate (20 mL) was added and the organic layer was dried over $MgSO_4$, filtered and evaporated to give **193** (0.26 g, quantitative) as white powder, mp 219 °C (dec). Rf baseline (EtOAc); ^{1}H NMR (DMSO-d_6, 90 °C, 400 MHz) δ_H 8.88 (1H, s, oxazolyl), 8.71 (1H, s, oxazolyl), 8.68 (1H, s, oxazolyl), 7.25 (5H, m, aryl), 5.28 (1H, m, C*H*CH_2, oxazolidinyl), 5.09 (1H, d, J_{HA} =16.4 Hz, C*H*HPh), 5.02 (1H, d, J_{HB} =12.6 Hz, CH*H*Ph), 4.34 (1H, dd, *J* =6.5, 9.3 Hz, C*H*H, oxazolidinyl), 4.15 (1H, dd, *J*=2.6, 9.3 Hz, CH*H*, oxazolidinyl), 1.69 (3H, s, CH_3), 1.56 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90°C, 100 MHz) δ_c 168.90 (HO-C=O), 163.45 (PhCH_2O*C*=O), 160.96 (s), 155.03 (s), 151.04 (s), 144.15 (d), 144.14 (d), 140.30 (d), 139.94 (s), 139.92 (s), 135.76 (s), 129.72 (s), 128.73 (d), 127.62 (d), 126.76 (d), 94.14 (N*C*(Me_2)O), 66.53 (OCH_2Ph, Cbz), 65.80 (OCH_2CH, oxazolidinyl), 54.02 (OCH_2*C*H, oxazolidinyl), 25.06 (CH_3), 25.02 (CH_3): HRMS calcd for $C_{23}H_{20}N_4O_8$ [M+H] 479.1202. Found 479.1224.

4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-tris oxazolyl benzyl ester (208)



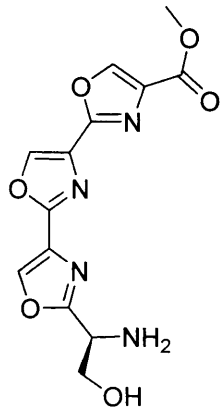
To a stirred solution of acid **193** (0.20 g, 0.42 mmol) in DMF (15 mL) was added triethylamine (1.3 mL, 10 mmol) and stirred at 20 °C for 10 min. The temperature was lowered to 0 °C and benzyl bromide (0.9 mL, 7.6 mmol) was added dropwise to the mixture and stirred to ambient temperature for 17 h. Then ethyl acetate (20 mL) was added and extracted with water (3x 20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **208** (0.21 g, 90 %) as white crystals, m.p 160-163 °C. $[\alpha_D]^{23}$ =-79.5 ° (*c*=1.0, CH_2Cl_2); IR (KBr) (ν_{max}) 3136, 1710, 1643, 1406, 1095, 698 cm^{-1}; Rf 0.2 (1:1 EtOAc: petroleum ether); ^1H NMR (DMSO-d_6, 90°C, 400 MHz): δ_H 8.92 (1H, s, oxazolyl), 8.88 (1H, s, oxazolyl), 8.67 (1H, s, oxazolyl), 7.42 (5H, m, aryl), 7.23 (5H, m, aryl), 5.37 (2H, s, C*H*$_2$OBn), 5.28 (1H, m, C*H*CH$_2$, oxazolidinyl), 5.12 (1H, d, J_{HA} =12.8 Hz, C*H*HPh), 5.00 (1H, d, J_{HB} =12.5 Hz, CH*H*Ph), 4.33 (1H, dd, *J* =6.5, 9.3 Hz, C*H*H, oxazolidinyl), 4.15 (1H, dd, *J*=2.6, 9.3 Hz, CH*H*, oxazolidinyl), 1.69 (3H, s, CH_3), 1.56 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 90°C, 100 MHz) δ_c 163.97 (PhCH$_2$-*C*=O), 160.21 (BnO-*C*=O), 155.60 (s), 155.01 (s), 145.46 (s), 140.87 (d), 140.83 (d), 140.70 (s), 140.66, 136.26, 135.78, 133.56 (s), 130.10 (s), 129.22 (d, aryl), 128.37 (d, aryl), 128,13, 127.97 (d, aryl), 127.63 (d, aryl), 127.26 (d, aryl), 94.65 (N*C*(Me$_2$)O), 67.03 (O=CO*C*H$_2$Ph), 66.30 (*C*H$_2$OBn), 66.16 (O*C*H$_2$CH,oxazolidinyl), 66.09 (OCH$_2$*C*H, oxazolidinyl), 25.53 (CH_3), 24.03 (CH_3): *Anal.* Calcd for $C_{30}H_{26}N_4O_8$: C, 63.15; H, 4.59; N, 9.82. Found: C, 62.71; H, 4.69; N, 9.60.

4-(2-Hydroxy-1-methoxycarbonyl ethyl carbomyl)-tris oxazolyl benzyl ester (209)



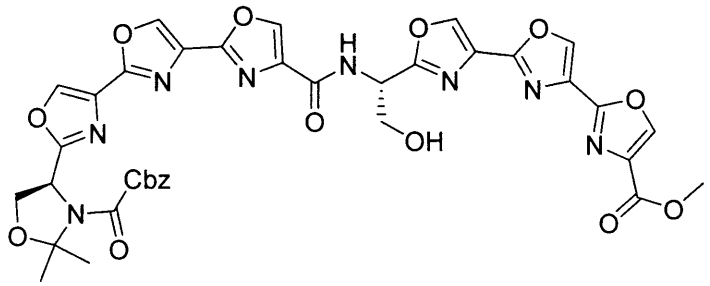
To a solution of ester **208** (0.10 g, 0.17 mmol) in dichloromethane/ methanol (25/ 5 mL) was added *p*-toluenesulfonic acid (0.03 g, 0.17 mmol) and stirred at reflux for 3 h. The mixture was evaporated and purified by column chromatography (1:3 ethyl acetate: petroleum spirit) to give **209** (0.09 g, 36%) as a white foam; $[\alpha_D]^{23}$ = -65.3 (*c*= 0.82, CH_2Cl_2); IR (thin film) (ν_{max}) 3296, 1724, 1699, 1544, 1153, 1109, 729 cm^{-1}; Rf 0.2 (EtOAc); ^1H NMR (DMSO-d_6, 90°C, 400 MHz): δ_H 8.90 (1H, s, oxazolyl), 8.88 (1H, s, oxazolyl), 8.82 (1H, s, oxazolyl), 7.37 (10 H, m, aryl), 5.37 (2H, s, C*H*$_2$OBn), 5.07 (2H, m, C*H*$_2$OPh), 4.88 (1H, m, C*H*CH$_2$OH), 3.83 (2H, m, CHC*H*$_2$OH); ^{13}C NMR (DMSO-d_6, 90°C, 100 MHz) δ_c 163.75 (PhCH$_2$-*C*=O), 159.79 (BnO-*C*=O), 155.36 (s), 154.61 (s), 150.70 (s), 145.08 (d), 145.03 (d), 140.25 (d), 140.22 (s), 136.44 (s), 135.38 (s), 133.13 (s), 129.64 (s), 127.99 (d, aryl), 127.96 (d), 127.79 (d), 127.78 (d), 127.68 (d), 127.66 (d), 66.08 (O=CO*C*H$_2$Ph), 65.77 (CH$_2$Ph), 61.47 (CH*C*H$_2$OH), 51.95 (*C*HCH$_2$OH),: LRMS m/z (EI) 530 (M^+, 74%), 337 (81%), 320 (19%), 301 (11%), 219 (18%), 105 (100%): HRMS calcd for $C_{30}H_{26}N_4O_8$ [M] 530.1437. Found 530.1466.

Amino alcohol-tris oxazolyl-methyl ester 179



A solution of oxazolidine **194** (0.06 g, 0.12 mmol) in methanol (10 mL) and dichloromethane (20 mL) was evacuated was then 10% palladium-on-carbon (6 mg, 0.001 mmol) was added. The mixture was evacuated and hydrogen was admitted. The mixture was stirred for 17 h and then evacuated. The mixture was then filtered through a pad of celite and the filtrate was evaporated to give **179** (0.030 g, 74%) as an orange oil, $[\alpha_D]^{23}$= -14.2° (*c*=1.0, CH_2Cl_2); IR (thin film) (ν_{max}) 3425, 1651, 1469, 1124, 617 cm^{-1}; Rf baseline (EtOAc); ^1H NMR (DMSO-d_6, 500 MHz) δ_H 9.05 (1H, s, oxazolyl), 9.03 (1H, s, oxazolyl), 8.91 (1H, s, oxazolyl), 4.02 (1H, m, C*H*CH_2OH), 3.83 (3H, s, OCH_3) 3.66 (2H, m, C*H*$_2$OH);^{13}C NMR (DMSO-d_6, 125 MHz) δ_c 167.76 (*C*=O), 160.87 (s, oxazolyl), 155.98 (s, oxazolyl), 154.97 (s, oxazolyl), 145.66 (d, oxazolyl), 140.83 (d, oxazolyl), 140.70 (d, oxazolyl), 133.28 (s, oxazolyl), 129.90 (s, oxazolyl), 128.62 (s, oxazolyl), 64.68 (*C*H_2OH), 51.98 (*C*HCH_2OH), 51.95 (OCH_3); LRMS m/z +(CI) 321 (M+H, 6%), 307 (12%), 289 (12%), 273 (7%), 244 (3%), 154 (100%): HRMS calcd for $C_{13}H_{12}N_4O_6$ [M+H] 321.0835. Found 321.0827.

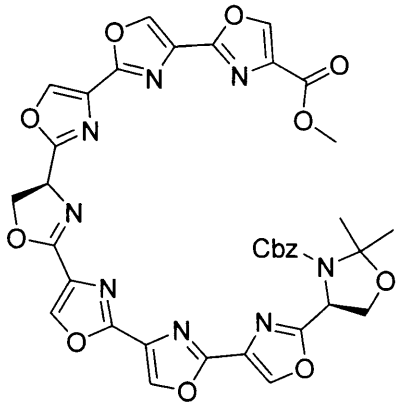
2-(1-Amino-2-hydroxy-ethyl)-trisoxazole-4-carboxylic acid methyl ester (Hydroxy-1-methoxycarbonyl ethyl carbomyl)-2,2-dimethyloxazolidine-trisoxazole (215)



To a solution of the acid **193** (0.19 g, 0.40 mmol) in DMF (80 mL) was added BOP (0.16 g. 0.44 mmol), DIEA (0.17 mL, 0.2 mmol) and HOBT (0.059 g, 0.44 mmol). The mixture was stirred for 10 min at -62 °C. Then a solution of amine **179** (0.13 g, 0.04 mmol) in DMF (10 mL) pre-cooled at -62 °C, was added to the mixture dropwise via a cannula. The mixture was left to stir for 96 h at -62 °C, then ethyl acetate (50 mL) was added and this mixture was washed with water (3x 50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified via column chromatography (ethyl acetate) to give **215** (0.12 g, 38%) as a white solid, mp 212 °C (dec); IR (ν_{max}) (KBr) 3456, 1724, 1658, 1350, 1099 cm^{-1}; Rf 0.1 (EtOAc); (DMSO-d_6, 80 °C, 400 MHz) ^1H NMR δ_H 8.91 (1H, s, oxazolyl), 8.87 (1H, s, oxazolyl), 8.85 (1H, s, oxazolyl), 8.77 (1H, s, oxazolyl), 8.72 (1H, s, oxazolyl), 8.67 (1H, s, oxazolyl), 8.51 (1H, d, *J*= 8 Hz, N-H), 7.24 (5H, m, aryl), 5.34 (1H, m, NC*H*CH_2OH), 5.03 (2H, m, CH_2Ph), 5.00 (2H, m, NCHC*H*$_2$OH), 4.33 (1H, m, oxazolidinyl), 4.15 (1H, m, oxazolidinyl), 3.86 (3H, s, OCH_3), 1.68 (3H, s, CH_3), 1.56 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 80 °C, 400 MHz); δ_c 163.56 (O-C=O), 161.67 (C=O), 160.36 (N-C=O) 155.34 (s), 155.18 (s), 154.25 (s), 151.11 (s), 150.45 (s), 149.72 (s), 144.79 (d), 142.70 (d), 140.77 (d), 140.46 (d), 140.24 (d), 140.07 (d), 135.87 (s), 133.12 (s), 129.87 (s), 129.69 (s), 128.83 (s), 128.69 (d), 127.75 (d), 127.26 (d), 126.87 (d), 94.23 (N*C*(Me_2)O), 66.63 (*C*H_2OCO, Cbz), 65.89 (O*C*H_2CH, oxazolidine), 58.70 (OCH_2*C*H, oxazolidine), 54.10(OCH_3), 51.27(*C*H_2OH), 49.57 (N*C*HCH_2O), 25.14 (CH_3), 23.66 (CH_3); LRMS m/z +(FAB) 783 (M+H,

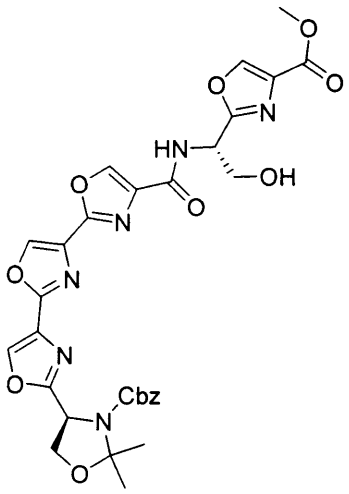
9%), 675 (5%), 481 (11%), 381 (2%), 338 (100%), 281 (4%), 207 (3%): HRMS calcd for $C_{36}H_{30}N_8O_{13}$ [M+H] 783.2010. Found 783.1996.

4-(2-Hydroxy-1-methoxycarbonyl ethyl carbomyl)-2, 2-dimethyloxazolidine-hepta-oxazolyl benzyl ester 216



To a stirred solution of amide **215** (0.11 g, 0.017 mmol) in anhydrous dichloromethane (10 mL) at -78 °C was added DAST (0.024 mL, 0.018 mmol) dropwise. The mixture was left to stir for 5 min and the solid was filtered and dried to give **216** (0.08 g, 80%) as a yellow solid, mp 289 °C (dec); IR (ν_{max}) (KBr) 3103, 1720, 1678, 1103, 985 cm^{-1}; ^1H NMR (DMSO-d_6, 120 °C, 400 MHz) δ_H 8.86 (1H, s, oxazolyl), 8.85 (1H, s, oxazolyl), 8.83 (1H, s, oxazolyl), 8.77 (1H, s, oxazolyl), 8.73 (1H, s, oxazolyl), 8.69 (1H, s, oxazolyl), 7.24 (5H, m, aryl), 5.67 (1H, m, NC*H*, oxazolidinyl), 5.28 (1H, m, CH_2CH, oxazolinyl), 5.12 (1H, d, *J* =12.7 Hz, OC*H*HPh), 5.01 (1H, d, *J* 12.7 Hz, OCH*H*Ph), 4.83 (2H, m, CH_2, oxazolinyl), 4.33 (1H, dd, *J* =6.5, 9.3 Hz, OC*H*H oxazolinyl), 4.13 (1H, dd, *J*=2.7, 9.3 Hz, OC*H*H oxazolinyl), 3.86 (3H, s, OCH_3), 1.72 (3H, s, CH_3), 1.57 (3H, s, CH_3); LRMS m/z +(FAB) 787 (M+Na, 27%), 705 (4%), 638 (3%), 484 (23%), 435 (7%), 329 (100%), 301 (15%); HRMS calcd for $C_{36}H_{28}N_8O_{12}$ [M+Na] 787.1724. Found 787.1728.

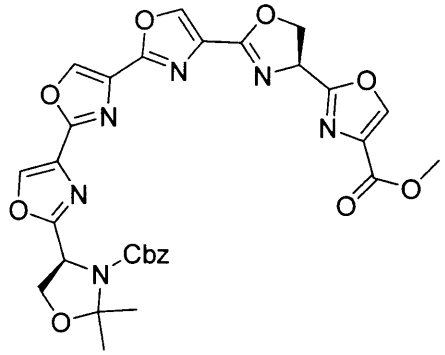
2-(1-Amino-2-hydroxy-ethyl)-oxazole-4-carboxylic acid methyl ester (hydroxy-1-methoxycarbonyl ethyl carbamoyl)-2, 2-dimethyloxazolidine-trisoxazole (224)



To a solution of the acid **193** (0.22 g, 0.46 mmol) in DMF (80 mL) was added BOP (0.22g. 0.50 mmol), DIEA (0.19 mL, 1.05 mmol) and HOBT (0.07 g, 0.52 mmol). The mixture was stirred for 10 min at -62 °C. Then a solution of the amine **105** (0.07 g, 0.38 mmol) in DMF (10 mL), pre-cooled at -62 °C was added to the reaction, dropwise via a cannula. The mixture was left to stir for 96 h at -62 °C, then ethyl acetate (50 mL) was added and this mixture was extracted with water (50 mL x3). The organic layer was dried over $MgSO_4$, filtered and evaporated to leave a residue which was purified via column chromatography (ethyl acetate) to give **224** (0.10 g, 40 %) as a colourless oil, $[\alpha]_D = -60.6$ (c=0.82, CH_2Cl_2); IR (ν_{max}) (thin film) 3446, 1720, 1653, 1598, 1409, 1350, 1097 cm^{-1}; Rf 0.1 (EtOAc); ^1H NMR (DMSO-d_6, 90 °C, 400 MHz); δ_H 8.91 (1H, s, oxazolyl *H*), 8.77 (1H, s, oxazolyl *H*), 8.70 (1H, s, oxazolyl *H*), 8.67 (1H, s, oxazolyl *H*), 8.36 (1H, d, J= 7.6 Hz, N-*H*), 7.25 (5H, m, aryl), 5.25 (2H, m, C*H*$_2$OH), 5.20 (1H, m, NC*H*CH$_2$OH), 5.13 (1H, d, J_{HA} =12.7 Hz, C*H*HPh), 5.05 (1H, d, J_{HB} =18.2 Hz, CH*H*Ph), 4.34 (1H, dd, J =6.8, 9.6 Hz, OC*H*H oxazolidinyl), 4.15 (1H, dd, J=6.4, 9.6 Hz, OCH*H* oxazolidinyl), 3.93 (1H, t, J =5.2Hz, O-*H*), 3.82 (3H, s, OC*H*$_3$), 1.69 (3H, s, C*H*$_3$), 1.54 (3H, s, C*H*$_3$); ^{13}C NMR (DMSO, 90 °C, 100 MHz); δ_c 163.99 (O-C=O), 162.96 (C=O), 160.99 (s), 159.56 (s), 155.60 (s),

154.39 (s), 145.20 (d), 142.33 (d), 140.86 (d), 140.62 (d), 136.57 (s), 136.26 (s), 132.49 (s), 130.10 (s), 129.20 (d), 128.14 (d), 127.64 (d), 127.25 (d), 94.63 ($NC(Me_2)O$), 67.05 (CH_2OCO, Cbz), 66.33 (OCH_2CH, oxazolidine), 54.45 (OCH_2CH, oxazolidine), 51.46 (OCH_3), 49.57 (CH_2OH), 42.63 ($NCHCH_2O$), 25.55 (CH_3), 24.05 (CH_3); LRMS m/z +(FAB) 671 (M+Na, 8%), 646 (7%), 592 (2%), 326 (15%), 281 (16%), 199 (19%), 176 (100%), 147 (48%): HRMS calcd for $C_{30}H_{28}N_6O_{11}$ [M+Na] 671.1713. Found 671.1699.

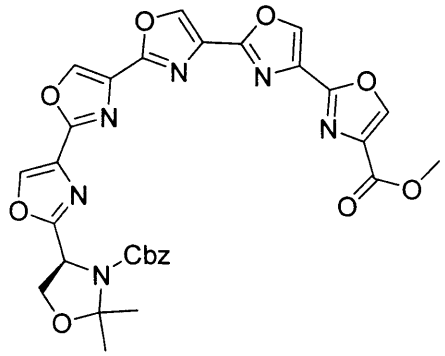
4-(2-Hydroxy-1-methoxycarbonylethyl carbamoyl)-2,2-dimethyloxazolidine-tris oxazole-oxazolinyl-oxazolyl methyl ester (223)



To a solution of amide **224** (0.11 g, 0.017 mmol) in anhydrous dichloromethane (10 mL) at -78 °C was added DAST (0.024 mL, 0.018 mmol) dropwise. The mixture was left to stir for 2.5 h and then potassium carbonate (0.03 g, 0.025 mmol) was added. After 17 h, water (10 mL) was added to the mixture and the resulting solution extracted with dichloromethane (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **223** (0.09 g, 85%) as a colourless oil, mp 236 °C (dec); IR(ν_{max}) (KBr) 3119, 1720, 1664, 1579, 1409, 1105, 985 cm^{-1}; Rf 0.2 (EtOAc); ^1H NMR (DMSO-d_6, 80 °C 400 MHz) δ_H 8.90 (1H, s, oxazolyl), 8.77 (1H, s, oxazolyl), 8.75 (1H, s, oxazolyl), 8.73 (1H, s, oxazolyl), 7.25 (5H, m, aryl), 5.60 (1H, m, NC*H*, oxazolidinyl), 5.27 (1H, m, CH_2CH, oxazolinyl), 5.12 (1H, d, J=12.8 Hz, OC*H*HPh), 4..80 (1H, d, J 12.8 Hz, OCH*H*Ph), 4.74 (2H, m, CH_2, oxazolinyl), 4.33 (1H, dd, J =6.5, 9.3 Hz, OC*H*H oxazolinyl), 4.14 (1H, dd, J=2.7, 9.3 Hz, OC*H*H oxazolinyl), 3.82 (3H, s, OCH_3), 1.68 (3H, s, CH_3), 1.57 (3H, s, CH_3); ^{13}C NMR (DMSO, 80 °C, 100 MHz) δ_c 163.92 (O-C=O), 163.22 (C=O), 160.82 (s), 159.02 (s), 155.60 (s),

155.17 (s), 151.49 (s), 145.55 (d), 144.95 (d), 142.70 (d), 140.68 (d), 140.43 (d), 136.23 (s), 132.64 (s), 130.60 (s), 130.16 (s), 129.28 (d), 128.08 (d), 127.58 (d), 127.24 (d), 94.66 (N*C*(Me$_2$)O), 70.35 (t, *C*H$_2$CH, oxazolinyl) 67.02 (*C*H$_2$OCO), 66.32 (OCH$_2$CH), 63.21 (t, CH$_2$*C*H, oxazolinyl), 54.41 (OCH$_2$*C*H, oxazolidinyl), 51.46 (OCH$_3$), 25.44 (CH$_3$), 23.99 (CH$_3$); LRMS m/z +(FAB) 653 (M+Na, 8%), 629 (1%), 413 (4%), 360 (56%), 326 (14%), 199 (28%), 176 (100%): HRMS calcd for $C_{30}H_{26}N_6O_{10}$ [M+Na] 653.1608. Found 653.1620.

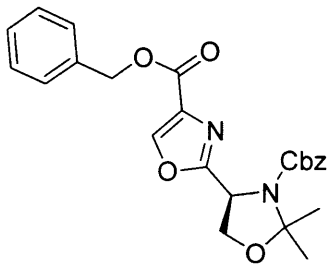
4-(2-Hydroxy-1-methoxycarbonylethylcarbamoyl)-2, 2-dimethyloxazolidine-penta-oxazolyl methyl ester (218)



To a solution of oxazoline **223** (0.06 g, 0.095 mmol) in anhydrous dichloromethane (30 mL) was added DBU (0.6 mL, 3.9 mmol). After 10 min bromotrichloromethane (0.2 mL, 1.0 mmol) was added dropwise at -10 °C. The mixture was left to stir for 30 min at 20 °C. The precipitate was filtered and washed with dichloromethane to give **218** as a yellow solid (0.04 g, 73%), mp 269 °C (dec); IR (ν_{max}) (KBr) 3113, 2341, 1717, 1649, 1512, 1406, 1095, 976 cm^{-1}; Rf 0.2 (EtOAc); ^{1}H NMR (DMSO-d$_6$, 313 K, 400 MHz): δ_H 8.99 (1H, s, oxazolyl), 8.97 (1H, s, oxazolyl), 8.90 (1H, s, oxazolyl), 8.85 (1H, s, oxazolyl), 8.78 (1H, s, oxazolyl), 7.24 (5H, m, aryl), 5.66 (1H, m, NC*H* oxazolidine), 5.30 (1H, dd, *J*=2.7, 6.5 Hz, NC*H*CH$_2$O), 5.14 (1H, d, *J* =12.7 Hz, C*H*HPh), 5.02 (1H, d, *J* =12.7 Hz, CH*H*Ph), 4.34 (1H, dd, *J* =6.5, 9.3 Hz, OC*H*H oxazolidine), 4.16 (1H, dd, *J*=2.7, 9.3 Hz, OC*H*H oxazolidine), 3.87 (3H, s, OCH$_3$), 1.70 (3H, s, CH$_3$), 1.57 (3H, s, CH$_3$); ^{13}C NMR (DMSO-d$_6$, 90 °C, 400 MHz) δ_c 163.35 (O-C=O), 160.09 (C=O), 155.09 (C=N), 154.95 (C=N), 154.87 (C=N), 154.31 (C=N), 150.96 (s), 144.30 (d), 140.12 (d), 140.05 (d), 140.00 (d), 135.68 (s), 133.09 (s), 129.67 (s), 129.62 (s), 129.53 (s), 128.72 (d), 127.46 (d), 126.97 (d),

126.66 (d), 94.13 (N*C*(Me$_2$)O), 66.43 (*C*H$_2$OCO), 65.79 (NHCH$_2$O), 53.96 (N*C*H CH$_2$O), 50.89 (OCH$_3$), 24.92 (CH$_3$), 23.57 (CH$_3$). LRMS m/z +(FAB) 651 (M+Na, 18%), 629 (1%), 553 (4%), 510 (3%), 360 (24%), 199 (23%), 176 (100%), 153 (72%): HRMS calcd for $C_{30}H_{24}N_6O_{10}$ [M+Na] 651.1452. Found 651.1462.

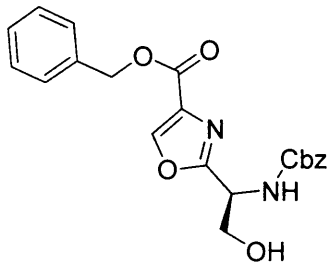
Benzyl 2-(3-(benzyloxycarbonyl)-2,2-dimethoxyloxazolidin-4-yl)oxazoles-4-carboxylate (228)



To a solution of acid **197** (0.33 g, 0.95 mmol) in DMF (15 mL) was added triethylamine (2.6 mL, 20 mmol); the mixture was stirred at 20 °C for 10 min. The temperature was lowered to 0 °C and benzyl bromide (1.81 mL, 15 mmol) was added dropwise. The mixture was allowed to warm up with stirring from 0 °C to ambient temperature over 17 h. Then ethyl acetate (20 mL) was added and extracted with water (3x 20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to give **228** (0.37 g, 90%) as a colourless oil, $[\alpha_D]^{23} = -12$ (*c*=1.0, $CHCl_3$); IR (thin film) (ν_{max}) 3418, 1720, 1585, 1454, 1226, 1068 cm^{-1}; Rf 0.4 (EtOAc); ^{1}H NMR (DMSO, 90°C, 400 MHz): δ_H 8.65 (1H, s, oxazolyl), 7.39 (5H, m, aryl), 7.19 (5H, m, aryl), 5.33 (2H, s, C*H*$_2$OBn), 5.21 (1H, m, C*H* oxazolidine), 5.10 (1H, d, J_{HA} =12.6 Hz, C*H*HPh), 4.98 (1H, d, J_{HB} =12.6 Hz, CH*H*Ph), 4.30 (1H, dd, *J* =6.4, 9.2 Hz, OC*H*H oxazolidine), 4.09 (1H, dd, *J*=6.4, 9.2 Hz, CH*H* oxazolidine), 1.65 (3H, s, CH$_3$), 1.54 (3H, s, CH$_3$); ^{13}C NMR (DMSO-d$_6$, 90 °C, 100 MHz) δ_c 163.33 (C=O), 160.28 (C=O), 151.46 (s), 145.37 (s), 136.24 (d), 135.89 (s), 132.62 (s), 128.35 (d), 128.13 (d), 128.02 (d), 127.88 (d), 127.64 (d), 127.22 (d), 94.61 (N*C*(Me$_2$)O), 67.00 (CH$_2$OBn), 66.26 (*C*H$_2$OCO, Cbz), 65.85 (OCH$_2$CH, oxazolidine), 54.44 (OCH$_2$*C*H, oxazolidine), 25.47 (CH$_3$), 23.99 (CH$_3$); LRMS m/z (EI) 436 (M$^+$, 14%), 421 (11%), 392 (1%),

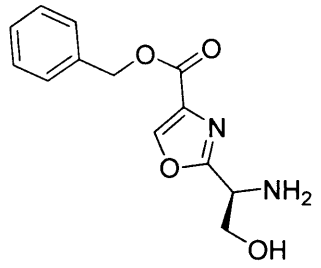
377 (87%), 239 (24%), 152 (15%), 91 (100%): HRMS calcd for $C_{24}H_{24}N_2O_6$ $[M]^+$ 436.1634. Found 436.1642.

Benzyl2-(1-(benzyloxycarbonylamino)-2-hydroxyethyl)oxazole-4-carboxylate (229)



To a solution of oxazolidine **228** (0.32 g, 0.73 mmol) in methanol (30 mL) was added *p*-toluenesulfonic acid (0.14 g, 0.73 mmol). The mixture was heated at reflux for 2.5 h. The mixture was then cooled to 20 °C, evaporated and the residue was purified via column chromatography (1:4 ethyl acetate: petroleum ether) to give **229** (0.15 g, 52%); as a colourless oil, $[\alpha_D]^{23}$ = -23.3 (*c*=1.0, $CHCl_3$); IR (thin film) (ν_{max}) 3290, 1724, 1699, 1544, 1261, 1068 cm^{-1}; Rf 0.2 (EtOAc); ^1H NMR (DMSO-d_6, 90 °C, 400 MHz) δ_H 8.69 (1H, s, oxazolyl), 7.35 (10H, m, aryl), 5.32 (2H, s, C*H*$_2$OBn), 5.05 (2H, m, C*H*$_2$O of Cbz), 4.82 (1H, m, C*H*CH$_2$OH), 3.77 (2H, m, C*H*$_2$OH); ^{13}C NMR (DMSO-d_6, 90 °C, 100 MHz) δ_c 163.20 (C=O), 160.04 (s), 155.25 (C=O), 144.93 (s), 136.42 (d), 135.45 (s), 132.04 (s), 127.96 (d), 127.77 (d), 127.63 (d), 127.52 (d), 127.23 (d), 127.06 (d), 65.44 (C*H*$_2$OBn), 65.34 (*C*H$_2$OCO, Cbz), 61.48 (OC*H*$_2$CH, oxazolidine), 51.48 (OCH$_2$*C*H); LRMS m/z +(CI) 397 (M+H, 6%), 307 (18%), 289 (12%), 273 (7%), 244 (3%), 154 (100%); HRMS calcd for $C_{21}H_{20}N_2O_6$ $[M+H]^+$ 397.1399. Found 397.1390.

Benzyl 2-(1-amino-2-hydroxyethyl)oxazoles-4-carboxylate (230)



A solution of carbamate **229** (0.11 g, 0.28 mmol) in methanol (20 mL) was evacuated and then 5% palladium-on-carbon (0.10 g, 0.69 mmol) was added. The mixture was evacuated and hydrogen was admitted into the reaction. The mixture was left to stir for 1.5 h and then evacuated. The resulting mixture was filtered through a pad of celite and the filtrate was concentrated to give **230** (0.06 g, 89%) as a colourless oil, $[\alpha_D]^{23}$ = -18.6 (*c*=0.87, $CHCl_3$); IR (ν_{max}) (thin film) 3570, 2916, 1633, 1361, 1080 cm^{-1}; Rf baseline (EtOAc); ^1H NMR (CD_3OD, 400 MHz) δ_H 8.28 (1H, s, oxazolyl), 7.27 (5H, m, aryl), 5.09 (2H, s, OCH_2), 4.92 (1H, t, *J* =3Hz, O*H*), 4.03 (1H, m, NH_2CH), 3.91 (2H, m, CH_2OH); ^{13}C NMR (CD_3OD, 100 MHz) δ_c 165.00 (C=O), 158.19 (s), 145.23 (s), 138.00 (d), 136.12 (s), 129.42 (d), 128.99 (d), 128.81 (d), 67.99 (CH_2OBn), 63.63 (CH_2OH), 53.17 (NH_2CHCH_2); LRMS m/z +(CI) 263 (M+H, 6%), 228 (7%), 199 (34%), 181 (3%), 91 (100%): HRMS calcd for $C_{13}H_{14}N_2O_4$ $[M+H]^+$ 263.1031, found 263.1035.

References

(1) Kelly, T. R.; Lang, F. R. *J. Org. Chem.* **1996**, *61*, 4623-4633.

(2) Faltin, C.; Fleming, E. M.; Connon, S. J. *J. Org. Chem.* **2004**, *69*, 6496-6499.

(3) Brand, M.; Drewes, S. E.; Loizou, G.; Roos, G. H. P. *Synthetic Commun.* **1987**, *17*, 795-802.

(4) Basavaiah, D.; Muthukumaran, K.; Sreenivasulu, B. *Synthesis* **2000**, 545-548.

(5) Monnin *Helv. Chim. Acta* **1958**, *41*, 2112-2119.

(6) Shin, C. G.; Okumura, K.; Ito, A.; Nakamura, Y. *Chem. Lett.* **1994**, 1305-1306.

(7) Reddy, K. V.; Jin, S. J.; Arora, P. K.; Sfeir, D. S.; Maloney, S. C. F.; Urbach, F. L.; Sayre, L. M. *J. Am. Chem. Soc* **1990**, *112*, 2332-2340.

(8) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1997**, *119*, 10587-10593.

(9) Nagano, H.; Hamana, M.; Nawata, Y.; Prachayasittikul, S.; Abdelsayed, A. N.; Bauer, L. *Heterocycles* **1987**, *26*, 1263-1270.

(10) Baker, A. T.; Singh, P.; Vignevich, V. *Aust. J. Chem* **1991**, *44*, 1041-1048.

(11) Deady, L. W.; Stanborough, M. S. *Aust. J. Chem.* **1981**, *34*, 1295-1302.

(12) Iwasa, S.; Nakamura, H.; Nishiyama, H. *Heterocycles* **2000**, *52*, 939-944.

(13) Pryor, K. E.; Shipps, G. W.; Skyler, D. A.; Rebek, J. *Tetrahedron* **1998**, *54*, 4107-4124.

(14) Endoh, N.; Tsuboi, K.; Kim, R.; Yonezawa, Y.; Shin, C. *Heterocycles* **2003**, *60*, 1567-1572.

(15) Chattopadhyay, S. K.; Biswas, S.; Pal, B. K. *Synthesis* **2006**, 1289-1294.

(16) Garner, P.; Park, J. M. *J. Org. Chemistry* **1987**, *52*, 2361-2364.

(17) Skinner, C. G.; Mccord, T. J.; Ravel, J. M.; Shive, W. *J. Am. Chem. Soc.* **1956**, *78*, 2412-2414.

(18) Shin, C. G.; Ito, A.; Okumura, K.; Nakamura, Y. *Chem. Lett.* **1995**, 45-46.

(19) Castro, B.; Dormoy, J. R. *Tetrahedron Lett.* **1972**, 4747-4750.

(20) Hoffmann, F.; Jager, L.; Griehl, C. *Phosphorus Sulfur and Silicon and the Related Elements* **2003**, *178*, 299-309.